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## Contents

*Viscosity Studies of Fluid Cream.* J. H. NAIR and D. E. MOOR ... 1

*Seasonal Effect on Yield of Dairy Cows.* C. Y. CANNON ... 11

*The Use of Corn Sugar in the Manufacture of Sweetened Condensed Skimmilk.* R. J. RAMSEY, P. H. TRACY, and H. A. RUEHE ... 17

*The Apparent and True Sugar of the Blood of Lactating Cows Fed Rations of Varying Fat Content.* KENNETH L. TURK and SAMUEL H. WORK ... 33

*Substances Adsorbed on the Fat Globules in Cream and Their Relation to Churning. II. The Isolation and Identification of Adsorbed Substances.* L. S. PALMER and HILDA F. WIESE ... 41

*The Leucocyte Content of Milk as Correlated with Bacterial Count and Hydrogen Ion Concentration for the Detection of Mastitis.* V. A. CHERRINGTON, H. C. HANSEN, and W. V. MALVERSEN ... 59

*Drying Up Cows and the Effect of Different Methods Upon Milk Production.* RALPH WAYNE, C. H. EURELL, and W. E. PETERSON ... 69

*The Effect of Various Methods For Drying Up Cows on the Bacterial and Cell Content of Milk.* RALPH WAYNE and H. MACY ... 79

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JOURNAL OF DAIRY SCIENCE

VOLUME XVI JANUARY, 1933 NUMBER 1

# VISCOSITY STUDIES OF FLUID CREAM*

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## INTRODUCTION

Since the average consumer generally judges the quality of fluid cream by the manner of its flow from a container, deeming the thickness of the cream a measure of its butterfat content, it is a matter of prime importance to the distributor of dairy products to maintain a uniformly heavy body in this product. In spite of constancy of method of cream handling and processing, there is considerable day-to-day variation in viscosity. It is desirable to have a simple method of judging cream body in the milk plant, both for checking the quality of the daily output and for investigating causes of observed fluctuations.

## PREVIOUS WORK

To date there has not been available any instrument sufficiently easy of manipulation by a creamery foreman or superintendent which gave any degree of certainty of results. Common practice has been to pour the cream from one container to another or to allow it to flow slowly down an inclined glass surface. Some have used an ordinary 50 cc. or 100 cc. pipette with indifferent success. These methods are inexact at best and the results obtained on different samples are scarcely comparable or reproducible.

Numerous studies of the viscosity of milk and cream have been made by various investigators, but the methods used have been largely empirical and more suitable for laboratory than for plant measurements. No generally accepted method has appeared. Dahlberg and Hening (5) used a MacMichael viscosimeter at temperatures of 3 to 4° C. Whitaker, *et al.*, (9) used Ostwald viscometers at various temperatures. Evenson and Ferris (6) used a 5 cc. pipette with capillary extensions 10 inches in length. They used various temperatures from 15° to 40° C. Sherwood and Smallfield (7) worked with a Scott and a Doolittle Torsion Viscometer at 17° C.

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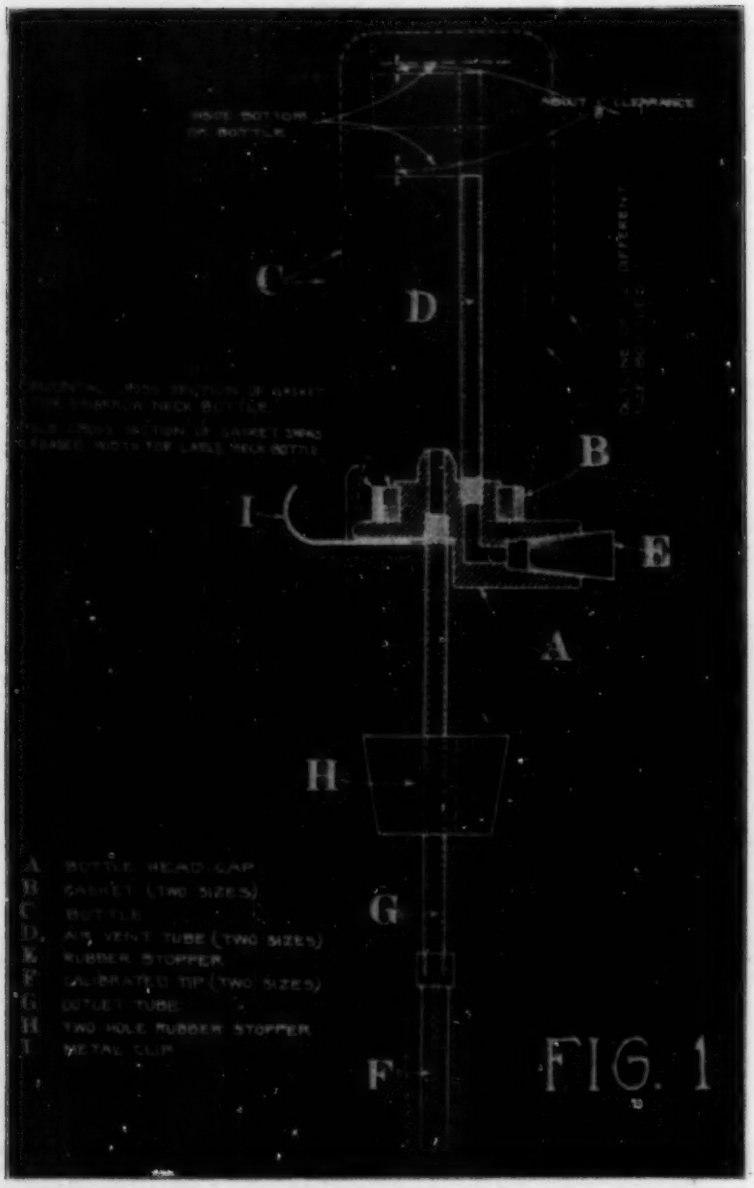

ABOUT ¼" CLEARANCE
C
D
B
I
E
A
H
G
F
A BOTTLE HEAD CAP
B GASKET (TWO SIZES)
C BOTTLE
D AIR VENT TUBE (TWO SIZES)
E RUBBER STOPPER
F CALIBRATED TIP (TWO SIZES)
G OUTLET TUBE
H TWO HOLE RUBBER STOPPER
I METAL CLIP

FIG. 1

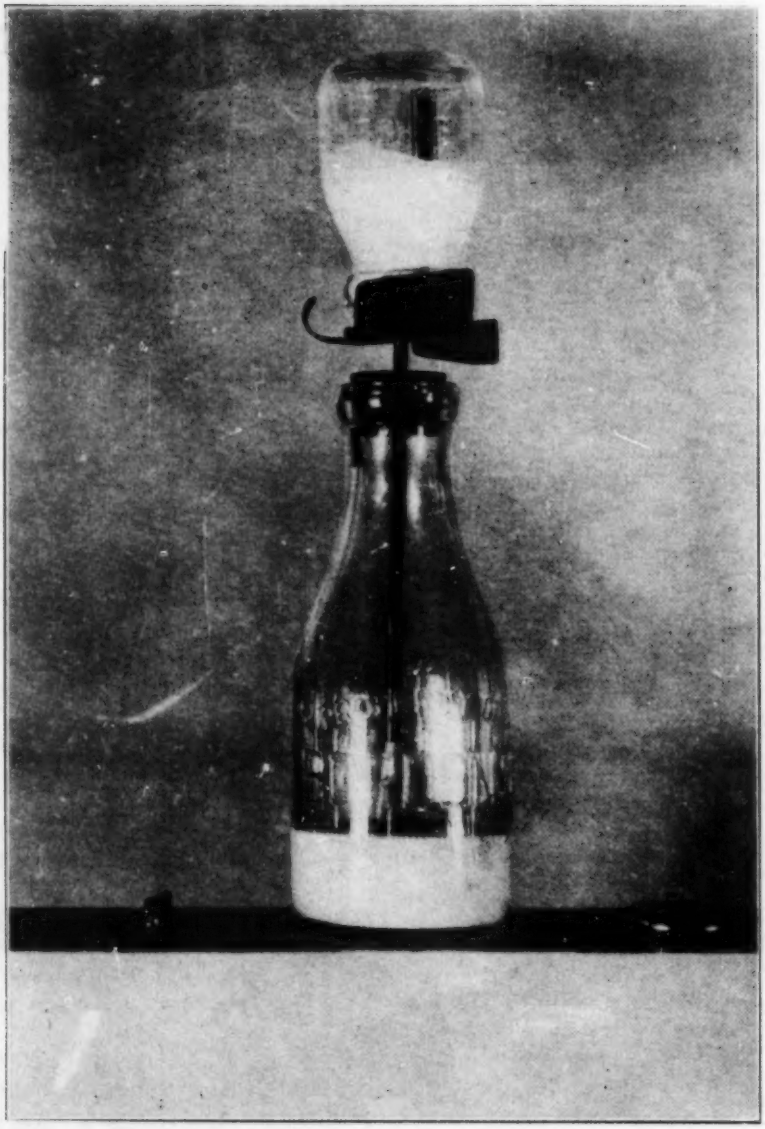
Bateman and Sharp (3) employed a Bingham viscometer, with thermostatic control at 25° C., while in another study the same authors (4) used a Bingham plastometer at the same temperature. Webb (8) used a 1 cc. capillary pipette with a diameter of 0.95 mm. and a length of 22.5 cm., making measurements at 20° C. Babcock (1) used a standard Saybolt viscometer in his recent extensive investigation of the viscosity of cream, expressing his data in terms relative to the viscosity of water taken as unity. This variation in instruments and temperatures together with frequent omission of data on composition and history of the cream makes a quantitative comparison and estimation of the results obtained extremely difficult.

## APPARATUS USED

Some eight years ago in this laboratory an extensive study of fluid cream, in which a MacMichael viscosimeter was used for viscosity measurements, led to conclusions largely in agreement with those subsequently published by Dahlberg and Hening (5). This work was presented before this Intersectional Meeting five years ago, but was never subsequently published. Recently, renewed interest on the part of production men caused the study to be reopened. The first requirement was an instrument which could be used in any dairy plant for determining cream body. A consideration of flow viscometers and some preliminary laboratory tests made this type appear most suitable for the purpose. Eventually a simple efflux instrument was developed and has been used in a number of plants for the past eighteen months. The design of this apparatus, which has been named the Borden Body Flow Meter, is shown in figure 1. The instrument is constructed of brass, with interchangeable tips. For creams containing 30 per cent or more of fat a tip approximately 2.78 mm. in diameter is used, while for thinner creams one with a diameter of approximately 1.97 mm. is used. The outlet tube projects into the bottle of cream sufficiently to insure the efflux of $222 \pm 0.5$ cc. of cream. The time required for the flow of this volume is recorded in seconds.

The operation of the flow meter is shown in the photograph, figure 2. The cream in the half-pint bottle is adjusted to temperatures chosen arbitrarily for commercial purposes; 15.6° C. is used for heavy creams and 10° C. for light creams. The flow meter, with the air vent tube closed by a cork stopper, is inserted into the bottle, air bubbles being carefully excluded, and the clip slid into place. The instrument is then inverted over a convenient receiver, usually a quart milk bottle, and supported in a vertical position (a No. 9 rubber stopper serves nicely). Upon removal of the air vent stopper, a stop-watch is punched and the time required for the flow of the entire volume is noted. Due to the heavy glass of which the bottles are constructed the change in temperature of the cream during

FIG. 2



a measurement is small, ½° C. or less. Typical flow data with a few liquids are shown in table 1.

TABLE 1
*Flow meter times of liquids*

| MATERIAL | TIP | TEMP. | FLOW TIME | ABSOLUTE VISCOSITY |
|---|---|---|---|---|
| | | °F. | *seconds* | *centipoises* |
| 60% Sucrose | Large | 15 | 286.5 | 74.9 |
| 40% Cream | " | 15 | 220.0 | ..... |
| Water | " | 15 | 24.5 | 1.145 |
| 60% Sucrose | " | 25 | 157.0 | 44.02 |
| 40% Cream | " | 25 | 138.0 | ..... |
| Stand. Oil | " | 25 | 107.0 | 79.0 |
| Water | " | 25 | 24.0 | 0.895 |

Observations made every 15 seconds on the volume of liquid discharged from the tip show that, with 60 per cent surose solution or 40 per cent cream, the rate is very nearly uniform over the entire range. The changing hydrostatic head apparently has a very slight effect, probably due to the fact that the diameter of the delivery tube is scarcely of capillary dimensions.

Application of Reynold's criterion (2) for the determination of existence of a turbulent régime in using the instrument gave calculated values with water of about 400. This is safely below the limit for transition from stream-line to turbulent flow. In no series of observations with this instrument were any turbulence or eddy effects noted.

## REPRODUCTIBILITY OF RESULTS

Thousands of measurements of creams of widely different viscosities have been made and correlated with known data on fat content, total solids, previous history, method of processing, size of fat globules and age. The expected concordance of results with given samples of cream is shown in table 2, giving data on ten separate portions of the same lots of 20 per cent and of 40 per cent butterfat creams, measurements having been made 24 hours after pasteurization.

## EFFECT OF TEMPERATURE

The viscosity of cream decreases rapidly with increasing temperature up to the melting point of the fat. Results obtained with the flow meter on the same cream measured at different temperatures are shown in table 3.

TABLE 2

*Variations in flow time in duplicate samples*

| | 20% CREAM SMALL TIP AT 10° C. | 40% CREAM LARGE TIP AT 15.6° C. |
|---|---|---|
| | *seconds* | *seconds* |
| Sample 1 | 88.5 | 192 |
| Sample 2 | 89.1 | 187 |
| Sample 3 | 88.8 | 181 |
| Sample 4 | 90.0 | 181 |
| Sample 5 | 91.0 | 190 |
| Sample 6 | 90.6 | 186 |
| Sample 7 | 89.8 | 190 |
| Sample 8 | 87.4 | 182 |
| Sample 9 | 91.2 | 183 |
| Sample 10 | 93.0 | 189 |
| Average | 90.0 | 186 |
| Standard deviation | 0.52 | 1.3 |

TABLE 3

*Effect of temperature on body flow time*

| TEMPERATURE | FLOW TIME OF | |
|---|---|---|
| | 20% PAST. CREAM | 40% PAST. CREAM |
| °C. | *seconds* | *seconds* |
| 10 | 94.7 | 261 |
| 15 | 85.9 | 195 |
| 20 | 79.0 | 131 |
| 25 | 71.0 | 88 |
| 30 | —— | 67 |
| 35 | —— | 52 |
| 40 | —— | 47 |

These observations are similar in trend to those reported by Babcock (1).

EFFECT OF BUTTERFAT CONTENT

Dahlberg and Hening (5) found that increasing the percentage of fat in cream always increased its viscosity, the effect being more pronounced in

TABLE 4

*Effect of butterfat content on flow time of pasteurized cream*

| BUTTERFAT | FLOW TIME AT 10° C. | BUTTERFAT | FLOW TIME AT 15.6° C. |
|---|---|---|---|
| *per cent* | *seconds* | *per cent* | *seconds* |
| 18 | 80.2 | 35.0 | 128.5 |
| 20 | 90.5 | 36.5 | 166.0 |
| 22 | 106.3 | 39.0 | 225.0 |
| 23 | 114.2 | 40.0 | 250.0 |
| 25 | 127.0 | 41.0 | 308.0 |
| 30 | 213.0 | 43.0 | 421.0 |

creams of higher fat content. Babcock (1) obtained similar results. Using the flow meter, with different tips for light and heavy cream as indicated, the following values were observed with increasing fat content:

Each series was made from a single lot of raw heavy cream, standardized before pasteurizing. It is to be noted that the higher the fat content the more pronounced is the effect of one per cent increases in fat content.

### AGING EFFECT

When fluid cream is stored in the cold a marked increase in viscosity occurs, a phenomenon commonly termed "aging effect." Cream, particularly if high in fat, appears to build up a gel-like structure which breaks down easily with agitation, but which re-forms on standing. A number of investigators (5), (4), (1) have attributed this phenomenon to the clumping of individual globules into large aggregates. While it remains to be proven that this is a true thixotropic phenomenon, it is certainly partly reversible. After the violent agitation accompanying separation, raw cream gradually becomes very viscous. Pasteurized cream is very thin in body when freshly cooled, but it increases rapidly during the first few hours of storage and more slowly for two or three days. Bateman and Sharp (4) have shown by their plastometer measurements that cream as low as 20 per cent in fat content has a definite yield value and that increasing fat content or aging of a given sample increases this value markedly. The aging effect can be followed very well with the flow meter.

TABLE 5

*The aging effect on raw and pasteurized cream*

| SAMPLE | BODY FLOW TIME AT 15.6° C. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fresh | 2 hr. | 4 hr. | 8 hr. | 16 hr. | 24 hr. | 48 hr. | 72 hr. | 96 hr. |
| Raw No. 1 | 123 | ..... | ..... | ..... | ..... | 151 | 156 | 158 | 160 |
| " " 2 | 132 | ..... | ..... | ..... | ..... | 151 | 180 | 205 | 206 |
| Past. No. 1 | 100 | 131 | 142 | 161 | 170 | 188 | 198 | 205 | 204 |
| " " 2 | 102 | 134 | 149 | 166 | 175 | 198 | 210 | 215 | 217 |

### SEASONAL AND TERRITORIAL VARIATION

Periodical observations of the flow time of light and heavy cream at different seasons of the year and at the same season in different sections of the

TABLE 6

*Seasonal and territorial variations in flow time of cream*

| SEASON | NEW YORK | | | CALIFORNIA | | | MIDDLE WEST | | | CANADA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20% | 24% | 39% | 20% | 24% | 39% | 20% | 24% | 39% | 20% | 24% | 39% |
| Summer | 77 | 106 | 140 | ..... | 118 | 135 | 86 | 104 | 155 | 84 | ..... | ..... |
| Winter | 87 | 123 | 175 | ..... | 125 | 140 | 86 | 110 | 205 | 92 | ..... | ..... |

United States and Canada have produced some interesting data. The seasonal differences show up more markedly in high butterfat creams.

In California the winter feeding conditions are quite different from those in other sections, being very similar to those prevailing in the East in summer. This may explain the low body in heavy cream produced there in winter. However, in one middle Western territory no seasonal differences were observed in the body of 20 per cent cream.

## VISIBLE DIFFERENCES IN BODY

It is important to correlate viscosity as measured with the flow meter with those differences in body which can be observed with the eye. To test this out, a number of people were asked to differentiate between paired samples of bottled cream of known viscosity, judging differences by a pouring test. Large differences were used in the initial pair of samples, this difference being gradually decreased in successive pairs until the point was reached where no observer could differentiate between samples. The data are too voluminous to be more than summarized here. With creams containing from 18 per cent to 25 per cent of fat, the average observer can always distinguish between creams differing by ten seconds in flow time and frequently detects differences of eight seconds. In heavy creams of 38 to 40 per cent fat content some observers can distinguish differences as small as 35 seconds in flow time. Most observers are certain to distinguish between creams 50 seconds different in flow time, while 60 seconds differences are always detected.

## SUMMARY

A relative viscometer of the gravity type which is suitable for the determination of the body of fluid cream in the ordinary milk plant test room has been devised and used to study conditions affecting the viscosity of cream.

The viscosity of cream is increased by lowering the temperature and by increasing the fat content.

Cream is not truly viscous, exhibiting thixotropic properties. With age, the body of cream increases rapidly for a few hours and more slowly for 48 hours. Agitation reduces the viscosity.

Considerable variation in the viscosity of fluid creams of the same fat content is found in different territories and at different seasons of the year.

A correlation between flow time of different creams and variations detectable by the average consumer has been worked out.

## ACKNOWLEDGMENT

The original suggestion of an efflux type of viscometer which was simple in design and adaptable to creamery plant use was offered by Dr. H. E. Van Norman.

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# SEASONAL EFFECT ON YIELD OF DAIRY COWS[1]

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Much attention has been recently given to the standardization of records so that comparisons can be made between the production of cows that have made their records under different conditions. The dairy cattle registry associations have recognized an effect of four factors on the production of cows and have set up classes which are governed by these factors in somewhat the same way. These factors are: (a) age of cow at beginning of test, (b) length of production period, (c) frequency of milking, and (d) the length of time during the lactation period the cow is carrying a calf.

Among other factors which influence production is that of the season in which the cow freshens. McDowell (2) analyzed the records of cows in 64 cow-testing associations from which he concluded that the cows freshening in the fall gave on the average a higher yield than the cows freshening at any other time. Those cows that freshened in the winter were second in yield while those cows that freshened in the spring and summer followed in order. He found that those cows that freshened in October gave the highest yield while those that freshened in June gave the lowest yield.

Wylie (5) concluded after studying the records of more than 2900 Register of Merit Jerseys that freshened in 1921 that the yearly fat production averaged the highest for cows which freshened in July, October, November, and December while those that freshened in April, May, and August gave the lowest average yield. He points out that the cows freshened in July gave the highest yield while the August freshened cows gave the lowest yield.

Sanders (3) (4) studied the effect of season on a large number of Norfolk (England) records. He found that cows freshening in October gave the largest yield of milk while the cows that freshened in June gave the smallest yield.

## EXPERIMENTAL

In order to determine the effect of seasonal changes found in Iowa on the yields of dairy cows, approximately 68,000 records taken from the Iowa Cow Testing Associations (Dairy Herd Improvement Associations), covering the years 1925–1930, were summarized. Only records of cows which were in the herd for a full year were used. There were cows of all the main dairy breeds represented in the group though more than one-half of the cows were Holsteins or Holstein grades.

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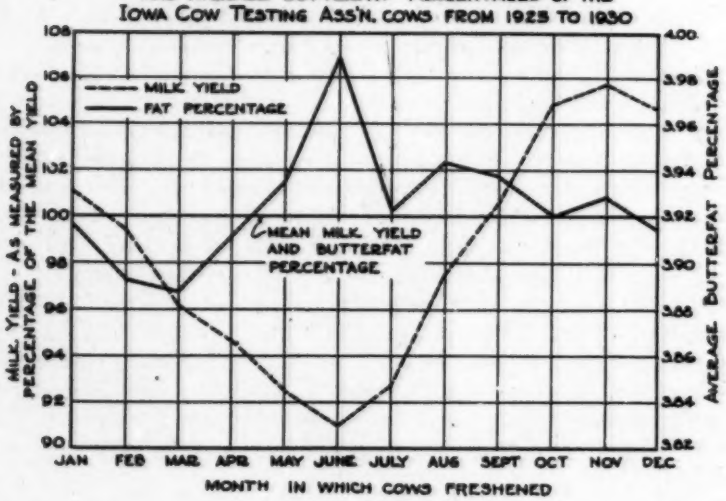
[1] Journal Paper No. B45 of the Iowa Agricultural Experiment Station.

Table 1 shows that the average production of the cows that freshened in November was the highest of any of the groups. There was a gradual decrease in average yield of the cows that freshened in each month until May. The groups that freshened in May and June and July all gave the same average yield of fat, though the June group yielded the smallest quantity of milk. From July to November each group showed a regular and consistent rise in yield.

The regularity of the curve in figure 1 shows the even fall and rise in milk yields of these groups. This regularity of month to month is quite different from that found by Wylie and Sanders.

FIGURE I

CURVES SHOWING TRENDS IN AVERAGE MILK YIELDS AND AVERAGE BUTTERFAT PERCENTAGES OF THE IOWA COW TESTING ASS'N. COWS FROM 1925 TO 1930

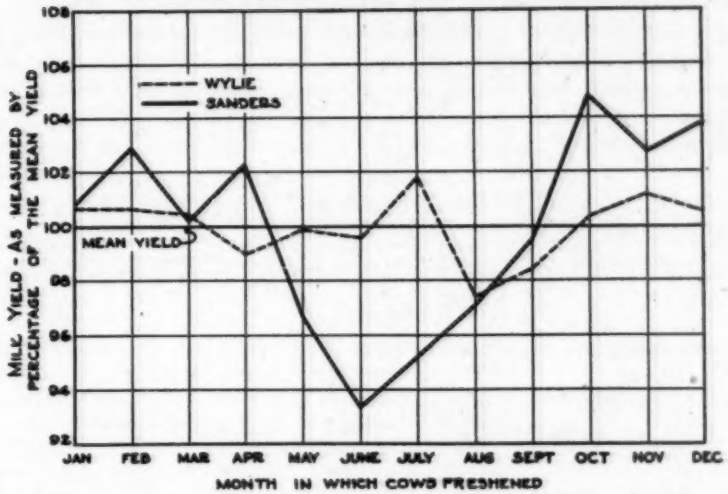



DISCUSSION

From the curves in figure 2 it can be seen that the cows studied by Wylie showed a great irregularity in their production for the group freshening in June gave a low yield while the July group gave the highest yield of any. This was followed by the lowest producing group (August) that Wylie studied. The very high yield of the July group would not be expected. The curve made from the Sanders records shows an expected regularity through the summer months but it exhibits irregularities through the winter months. Sanders (4) suggests that "with a very large mass of data the curve might rise sharply at October and then fall in approximately a straight line to April, or it might be rounded giving a maximum at December." In his studies with Hammond (1) where more records were used

FIGURE II

CURVES SHOWING TRENDS IN AVERAGE MILK YIELDS OF COWS REPORTED BY WYLIE AND SANDERS




the curve was somewhat smoothed out. One explanation of these irregularities is that the sample of cows freshening in some of the months may have been, just by chance, higher producing cows than those freshening in other months.

The lack of smoothness in Wylie's curve might be caused by his using only cows that freshened in a single year when seasonal changes might vary widely from an average of several years. The data collected from the Iowa Cow Testing Associations would not be subjected to these criticisms because the number of records involved is large and the records were made over a period of years. That this is true is evident from the smoothness of the curve (Fig. 1) based on these data.

Evidently the season of the year at which a cow freshens has an influence on her year's total production. This influence is probably not due to climate alone but to management also. Besides the direct effects of climate on a cow there is an indirect effect on milk yield because climate modifies the feed crops which she consumes. Some of this effect may be influenced by the use of soiling crops or some other change in management.

Probably this accounts for the levelness of the curve calculated from Wylie's figures on Register of Merit Jerseys. There was much less difference between his low and high groups than in the Iowa groups. This levelness of yields indicates that feed and care are important factors in determining the yields of the groups. These cows were undoubtedly fed on a higher level than the Iowa cows.

### BUTTERFAT PERCENTAGES

A study of the average butterfat percentages of the milk yielded by the various groups in the Iowa Cow Testing Associations shows little fluctuation. The milk of the group which freshened in June contained the highest percentage of fat, enough higher than the milk of both May and July groups so that even though the latter two groups had larger milk yields the fat yields of all three were the same. The statistical significance of this was not tested.

### CORRECTION FACTORS

In order to standardize these records to a common basis, correction factors were calculated using the mean yield of all the records as a standard (table 1). These factors include not only the direct effects of Iowa cli-

TABLE 1

*Average yearly yield of milk and butterfat together with correction factors for each group freshening each month*

| MONTH | NUMBER OF RECORDS | AVERAGE YEARLY MILK PER COW POUNDS | CORRECTION FACTOR FOR MILK YIELD | AVERAGE PER CENT OF FAT | AVERAGE YEARLY BUTTERFAT YIELD PER COW POUNDS | CORRECTION FACTOR FOR FAT YIELD |
|---|---|---|---|---|---|---|
| January | 7049 | 7453 | 0.99 | 3.92 | 292 | 0.99 |
| February | 6181 | 7326 | 1.01 | 3.90 | 285 | 1.01 |
| March | 6192 | 7088 | 1.04 | 3.89 | 276 | 1.05 |
| April | 4563 | 6971 | 1.06 | 3.92 | 273 | 1.06 |
| May | 3621 | 6810 | 1.08 | 3.94 | 268 | 1.08 |
| June | 2753 | 6705 | 1.10 | 3.99 | 268 | 1.08 |
| July | 2784 | 6835 | 1.08 | 3.92 | 268 | 1.08 |
| August | 3400 | 7191 | 1.02 | 3.95 | 284 | 1.02 |
| September | 7493 | 7399 | 1.00 | 3.94 | 292 | 0.99 |
| October | 8094 | 7732 | 0.95 | 3.92 | 304 | 0.95 |
| November | 8202 | 7798 | 0.94 | 3.93 | 307 | 0.94 |
| December | 7660 | 7722 | 0.95 | 3.92 | 303 | 0.95 |
| Totals and Averages | 67992 | 7372* | | 3.92 | 289* | |

* These figures are weighted means.

mate on cows but also the seasonal variations in feeding and management conditions found in the Iowa Cow Testing Associations. These conditions are somewhat similar throughout the North Central States, so these factors would probably give accurate results in correcting records made in this area for seasonal effects.

SUMMARY

A study of nearly 68,000 Iowa Cow Testing Associations yearly records shows that the group which freshened in the month of November had the highest average yield. From November to June the group freshening in each month produced less than the group freshening the preceding month. From June until November the group freshening in each month had a larger production than the group of the preceding month. Although June showed the smallest average yield of milk, its butterfat yield was the same as that of the May and July groups.

Factors were calculated from these averages based on the mean yield of all the records. These factors can be used in correcting yields where the cows started in different months so as to reduce them to a common standard.

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# THE USE OF CORN SUGAR IN THE MANUFACTURE OF SWEETENED CONDENSED SKIMMILK[1]

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Corn sugar or dextrose is a monosacchoride prepared by the hydrolysis of corn starch as represented by the general equation: $(C_6H_{10}O_5)n + nH_2O = n(C_6H_{12}O_6)$. A more extensive use of this product has been made possible by a ruling of the Secretary of Agriculture (1) under date of December 26, 1930, which states in part—"the use of pure refined corn sugar as an ingredient in the packing, preparation or processing of any article of food in which sugar is a recognized element need not be declared upon the label of any such product." This ruling has added new impetus to studies of the use of corn sugar in manufactured food products.

The possibility of using corn sugar in sweetened condensed skimmilk is of considerable economic importance since sucrose (cane or beet sugar) forms a large proportion of the cost of the finished product. As usually manufactured, sweetened condensed skimmilk contains 28 per cent skimmilk solids and 40–44 per cent sucrose, which is added for its preservative effect.

Since both condensed skimmilk and corn sugar are used extensively in the manufacture of bread, there should be no objection from the baking industry to a combination of corn sugar and milk at the condensery. Experiments conducted at a commercial bakery showed that dough containing condensed skimmilk sweetened with corn sugar, as compared with one containing cane sugar, produced bread that was of better texture and toasting quality, and equally as good in flavor.

In a study conducted by Morrison and Gerber (2) at the American Institute of Baking, it was found that when cerelose was substituted for cane sugar in the dough there was no appreciable difference in the total fermentation period. The judges could detect no difference in the appearance of the experimental breads. The total score of the bread made with 4.5 per cent cerelose was 94.5 as compared with a total score of 92 for the product containing 4.5 per cent cane sugar. This difference was due to a slightly higher score for grain, color of crumb, and texture. No difference in flavor was reported. It would seem from these studies, therefore, that the presence of cerelose in sweetened condensed skimmilk would not detract from the quality of the bread, but may even result in certain advantages.

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[1] An abstract of a Master's Thesis submitted by R. J. Ramsey as partial fulfillment of the requirements for the degree of Master Science in Dairy Husbandry in the Graduate School of the University of Illinois, 1932.

Sweetened condensed skimmilk is sometimes used as a source of concentrated skimmmilk solids in the manufacture of ice cream. Inasmuch as corn sugar has been sometimes used by this industry to replace approximately 25 per cent of the cane sugar in the mix, it is not likely that any objections would be made by the ice cream manufacturer to the presence of corn sugar in the condensed skimmilk. It might seem, therefore, that the success of such a product would depend upon whether or not it had as good keeping qualities as condensed milk sweetened with cane sugar. However, preliminary experiments conducted on a commercial scale in the University of Illinois Creamery showed that certain factors other than keeping quality must be considered. Condensed skimmilk sweetened with corn sugar was found to be objectionable because of the brown discoloration, physical thickening, and mass crystallization of the corn sugar that occurred during storage.

Since the use of corn sugar is of sufficient economic importance to merit further consideration, this project was designed to determine: (1) the preserving power of dextrose, (2) the properties of dextrose which are responsible for the defects incident to its use, (3) the possibilities of changing the methods of processing the condensed skimmilk to make the use of corn sugar possible, and (4) the extent to which corn sugar could be substituted for sucrose.

## THE MICROBIOLOGY OF CONDENSED SKIMMILK SWEETENED WITH CORN SUGAR

Bacteriological analyses of different lots of sweetened condensed skimmilk indicated that the types of organisms encountered in sweetened condensed skimmilk are the same, regardless of the kind of sugar used. The predominating flora was found to consist primarily of micrococci and large aerobic spore formers of the subtilis-mesentericus group.

The principal types of bacterial spoilage found in condensed skimmilk sweetened with corn sugar consisted of "bloats," "lumps," and "thickened." Other investigators (3, 4, 5, 6, 7, 8, 9) have reported similar observations on products containing sucrose. It seems likely that bacterial thickening does not depend wholly upon the acid formed. The existing data are insufficient to warrant drawing any definite conclusions as to the rôle of bacterial rennin or similar enzymes. The present method for determining rennin formation in milk was found to be unreliable.

Preliminary experiments on the bacteriology of sweetened condensed skimmilk demonstrated that it was difficult to make an accurate analysis of this viscous, candied product. Many organisms grew in clumps, thus making it difficult to secure an accurate sample. It was impossible to make pure culture studies, since the condensed milk could not be sterilized without coagulation and destruction of the sugar. It was evident, therefore,

that sweetened condensed skimmilk was not a desirable medium in which to compare the preserving power of sugars.

In order to facilitate culture studies, solutions of broth were used. To prepare the solutions, sufficient sugar (anhydrous) was added to the plain broth to give the desired concentration. The solution was then standardized to a pH of 6.8 and sterilized. Pure cultures were then inoculated into the sterile sugar solutions of various concentrations in plain broth, and these were incubated at 100° F. The dilutions were made with a pipette.

It was disappointing to find that even this method did not give desirable results in all cases because of the destruction of the sugar and the formation of acid during sterilization. The condensation of the aldehyde and ketone radicals of the sugars with free amino groups probably hastened this reaction. Dextrose solutions usually decreased from pH 6.4 to about pH 5 while sucrose solutions changed only from pH 6.8 to pH 6.0. In 1917 Mudge (10) made similar observations, which were later confirmed by McAlpine (11) in 1923.

The procedure was modified by sterilizing the plain broth and later adding the sugar to give the desired concentration. Changes in pH were avoided in this manner, but contamination caused some difficulty.

The solutions used to secure the data presented in table 1 were prepared by adding sugar to concentrated sterile broth. The results shown in table 2 were obtained by using sterile sugar broth solutions.

## PRESERVING PROPERTIES OF SUGARS

Theoretically the preserving power of dextrose is almost twice as great as that of sucrose, provided the preserving power is directly proportional to the osmotic pressure. However, numerous experiments demonstrated that in concentrated solutions dextrose was only slightly more efficient than sucrose as a preserving agent. Typical data presented in tables 1 and 2 show that at low concentrations sucrose may exert more preserving power

TABLE 1

*The comparative bacteriological efficiency of dextrose and sucrose in plain broth solutions inoculated with a large spore-forming rod isolated from sweetened condensed milk. (Incubated at 100° F.)*

| PERCENTAGE SUGAR | PH OF MEDIA | INOCULATION | COUNT AT THE END OF 14 DAYS |
|---|---|---|---|
| 40 dextrose (anhydrous) | 6.3 | 10,000 | Spoiled |
| 50 " " | 6.2 | " | 300,000 |
| 65 " " | 6.0 | " | 1,800 |
| 40 sucrose | 6.8 | " | Spoiled |
| 50 " | 6.7 | " | 200,000 |
| 65 " | 6.6 | " | 42,000 |

than dextrose and at high concentrations dextrose and sucrose may have approximately the same preserving power.

TABLE 2

*The bacteriological efficiency of dextrose and sucrose in plain broth solutions inoculated with a large spore-forming rod isolated from sweetened condensed milk. (Incubated at 100° F.)*

| PERCENTAGE SUGAR | PH OF MEDIA | INOCULATION | COUNT AFTER 6 DAYS |
|---|---|---|---|
| 20 sucrose | 6.0 | 1,000,000 | 6,640,000 |
| 30 " | 5.6 | " | 6,450,000 |
| 40 " | 5.7 | " | 6,400,000 |
| 50 " | 5.8 | " | 320,000 |
| 18.2 dextrose (anhydrous) | 5.5 | " | 14,000,000 |
| 27.3 " " | 5.1 | " | 16,000,000 |
| 36.4 " " | 5.0 | " | 600,000 |
| 45.5 " " | 5.0 | " | 580,000 |

It was evident, therefore, that one of three conclusions must be drawn. Either dextrose does not exhibit the osmotic pressure that has been attributed to it; or sucrose exhibits an abnormally high osmotic pressure; or the preserving power of the sugar depends on some other property in addition to its osmotic pressure.

The works of Morse (12), Collander (13), Findlay (14), Berkeley (15), and Frazier (16) have shown that neither dextrose nor sucrose exhibits its calculated osmotic pressure. (The osmotic pressure is calculated on the basis of gram-molecular volume, *i.e.*, one gram molecule of sugar per liter of solution should give an osmotic pressure of 22.4 atmospheres (15)). The relative osmotic pressure values of dextrose and sucrose are shown in tables 3 and 4, which were constructed from the observations of Berkeley (15). These data show that as the concentration is increased the efficiency of sucrose gradually approaches that of dextrose. The variations between calculated and observed osmotic pressures are evidently due to hydration of the sugar in solution. In solution sucrose apparently forms a pentahydrate (13), thereby exaggerating the osmotic pressure. Dextrose probably reacts with only two molecules of water. Therefore, as the concentration is increased the difference in osmotic pressure between equi-molecular solutions of sucrose and dextrose is greatly increased. In sweetened condensed skimmilk the osmotic pressure of sucrose is about 185 atmospheres (16), almost four times its theoretical osmotic pressure in an ideal solution. This observation no doubt explains why sucrose exhibits almost as much osmotic pressure as dextrose pound for pound. Other minor factors which may influence the osmotic pressure are the inversion of the sucrose, and the water content (approximately 10 per cent) of corn sugar.

TABLE 3

*The osmotic pressure of dextrose and sucrose solutions* (15)

| PERCENTAGE SUGAR (ESTIMATED) | OSMOTIC PRESSURE (ATM.) | CALCULATED OSMOTIC PRESSURE FOR IDEAL SOLUTIONS | GRAMS OF SUGAR PER LITER OF SOLUTION |
|---|---|---|---|
| | | *Dextrose* | |
| 9.6 | 13.21 | 11.32 | 99.8 |
| 20.5 | 29.17 | 25.76 | 199.5 |
| 28.6 | 53.19 | 39.64 | 319.2 |
| 38.7 | 87.87 | 55.77 | 448.6 |
| 45.9 | 121.18 | 68.09 | 548.6 |
| | | *Sucrose* | |
| 16.9 | 13.95 | 11.80 | 180.1 |
| 26.5 | 26.77 | 19.64 | 300.2 |
| 36.3 | 43.97 | 27.32 | 420.3 |
| 44.9 | 67.51 | 35.39 | 540.4 |
| 53.0 | 100.78 | 43.00 | 660.5 |
| 58.7 | 133.74 | 49.05 | 750.6 |

TABLE 4

*The relation of concentration to the relative osmotic pressure exerted by dextrose and sucrose*

| OSMOTIC PRESSURE | PERCENTAGE RATIO OF DEXTROSE TO SUCROSE |
|---|---|
| 10 | 1: 1.60 |
| 20 | 1: 1.451 |
| 40 | 1: 1.437 |
| 60 | 1: 1.400 |
| 80 | 1: 1.314 |
| 100 | 1: 1.276 |
| 120 | 1: 1.252 |

The data presented in table 2 are interesting, since they show that sugar solutions, which would be expected to have about the same osmotic pressure, varied greatly in preserving power. Apparently, some other explanation in addition to osmotic pressure must be advanced to explain the differences in preserving power. It seems possible that the metabolism of the organism may be an important factor in this respect.

## CRYSTALLIZATION OF DEXTROSE IN SWEETENED CONDENSED SKIMMILK

One of the difficulties encountered in the use of dextrose in the manufacture of sweetened condensed skimmilk was the mass sugar crystallization that occurred during storage. As shown by the data in table 5 (constructed from the data presented by Jackson and Silsbee (17) and Browne

(18)), dextrose is less soluble than sucrose, especially at low temperatures. It seemed advisable, therefore, to replace only a part of the sucrose with corn sugar. The results of some of the combinations tried are shown in table 6. Although it was found possible to replace approximately 75 per cent of the sucrose with corn sugar without getting crystallization after 130 days' storage at 40° F., it was thought advisable to replace only 50 per cent of the sucrose with corn sugar. In this way danger of crystallization of dextrose would be eliminated. Several commercial batches of sweetened condensed skimmilk containing half corn sugar and half cane sugar were made and marketed. There was no crystallization of dextrose in this product after three months of storage at 40° F.

TABLE 5

*The solubility of sucrose and dextrose in water* (17, 18)

| °C. | °F. | PER CENT SUGAR IN WATER SOLUTIONS | |
|---|---|---|---|
| | | DEXTROSE | SUCROSE |
| 0 | 32 | 30.8 | |
| 10 | 50 | 40.8 | 65.58 |
| 15 | 59 | 44.0 | 66.30 |
| 20 | 68 | 47.2 | 67.09 |
| 25 | 77 | 50.3 | 67.89 |
| 30 | 86 | 54.64 | 68.70 |
| 35 | 95 | 58.02 | 69.55 |
| 40 | 104 | 61.87 | 70.42 |
| 45 | 113 | 65.71 | 71.32 |
| 50 | 122 | 70.91 | 72.25 |

TABLE 6

*The crystallization of dextrose from sweetened condensed skimmilk stored at 40° F.*

| PERCENTAGE SUGAR (DRY WEIGHT) | PERCENTAGE IN WATER | DAYS REQUIRED FOR CRYSTALS TO APPEAR |
|---|---|---|
| Dextrose 22.75 | 31.6 | Smooth after 90 days |
| " 27.30 | 37.9 | " " " " |
| " 31.85 | 44.3 | 75 (very many crystals) |
| " 38.22 | 53.0 | 16 " " " |
| " 22.75 + sucrose 17 | 55.1 | Smooth after 90 days |
| " 27.3 + " 12 | 54.5 | " " " " |
| Sucrose 42 | 58.3 | " " " " |
| Dextrose 22.75 + glucose sirup 16 | 39.3 | 56 |

## THE PHYSICAL THICKENING OF SWEETENED CONDENSED SKIMMILK

One of the outstanding defects encountered in condensed skimmilk sweetened with dextrose was the progressive thickening during storage at high temperatures. This increase in viscosity was not accompanied by the

formation of the disagreeable odor characteristic of sweetened condensed milk thickened by bacteria.

This physical thickening was found to be due to the effect of dextrose on the stability of the casein and albumin, especially the former. The exact nature of this reaction has not been determined, but the evidence available indicates a direct reaction between the protein and the dextrose. When proteins were heated with dextrose they lost most of their Biuret reaction, a fact which suggests an amino-aldehyde condensation.

During this experiment additional evidence has been revealed to indicate that sugars react with proteins directly. Various sugars were heated with skimmilk as well as freshly precipitated casein. This heating process was accompanied by the formation of a dark brown color when dextrose, lactose, or levulose were used. The color was absorbed by the protein material. The color is probably an amino acid-sugar condensation product, the ketone or aldehyde radical being attached to the amino acid. The casein apparently reacted with the sugar, for it lost most of its Biuret reaction. Prolonged heating of skimmilk containing corn sugar resulted in a change in the physical nature of the casein. At times it was difficult or even impossible to precipitate the proteins with acids or rennet. Even hydrochloric acid would not precipitate the casein without vigorous boiling. When precipitated, the curd particles were very small. In cold solutions the acid merely increased the viscosity. The curd formed with rennet and $CaCl_2$ was very soft and flocculent. The addition of some mineral salts hastened coagulation with acids. It was also interesting to note that dextrose would stabilize the casein of unsweetened condensed milk autoclaved at 240° F.

The fact that casein loses most of its Biuret reaction during heat treatment with dextrose indicates that at least part of the action of the dextrose is directly concerned with the protein. It seems probable that the aldehyde radical of the dextrose attaches itself to some of the amino groups in the casein molecule. This view is further substantiated by the fact that such casein gives a strong reducing test with Benedict's solution. During this experiment several samples of casein were made from different lots of skimmilk to some of which dextrose had been added. At temperatures as low as 40° F. the casein absorbed or in some way attached itself to the dextrose molecule, as shown by the abnormal or gray appearance of the casein. This casein was very brown after drying at 145° F., while normal casein was only slightly discolored.

When skimmilk and dextrose were heated together their affinity for each other increased. As the heating temperature was raised the sugar became so firmly attached to the casein that no amount of washing would remove it. In other words, the Benedict test became stronger as the heating

temperature was increased. There is also a possibility that the sugar may react with the mineral salts in such a way as to effect the stability of the protein. Browne (18) has shown that sugars react with minerals to give basic sugar compounds.

$$\underset{\text{salt}}{MA} + \underset{\text{dextrose}}{C_6H_{12}O_6} + \underset{\text{water}}{H_2O} = \underset{\substack{\text{basic glucose}\\\text{compound}}}{MOH \cdot C_6H_{12}O_6} + \underset{\substack{\text{ionized}\\\text{acid}}}{HA}$$

The physical thickening may be avoided to a great extent by forewarming the milk and sugar separately. High forewarming temperatures increase the tendency to thicken. If the milk and sugar are forewarmed separately the forewarming temperature ceases to be of any importance as far as corn sugar is concerned. However, the lactose in the milk will alter the stability of the protein if excessive forewarming temperatures are used. The sugar should be added in a concentrated solution at the end of the condensing period.

### THE BROWN DISCOLORATION OF SWEETENED CONDENSED SKIMMILK

The natural color of fresh milk is almost impossible to secure in condensed milk products because of the brown discoloration that occurs during processing and storage. This defect, which makes such products less attractive and even unsalable at times is especially apparent in sweetened condensed milk in which corn sugar has been substituted for cane sugar.

Hunziker (6) and Savage and Hunwicke (19) state that the brown discoloration of sweetened condensed milk is due to the caramelization of the sucrose and lactose, especially the former. Hunziker also states that the proteins may be darkened by excessive heating.

*The Caramelization of Sugars.* Experiments with corn sugar demonstrated that it caused a marked browning of sweetened condensed skimmilk. Since similar products made with cane sugar were far superior in color, an experiment was designed to determine the relative sensitivity of corn sugar, cane sugar, and milk sugar towards caramelization. Water solutions of sugars failed to show any evidence of caramelization when heated to 250° F. for thirty minutes. Pure white albumin and casein also failed to darken when heated in distilled water solution. However, when milk, albumin, or casein were heated with lactose or dextrose there was a marked brown discoloration. These observations indicated that perhaps the brown discoloration of sweetened condensed milk and evaporated milk should be explained on some other basis than caramelization.

Leeds (20), who worked on the effect of sterilization on milk, says: "The decomposition product of the milk sugar is the chief substance concerned in the development of the brown color, but not entirely, since the mixture of casein and albumin separated from such heated milk and treated

with ether to remove the fat, has, on drying, a strong yellow color. The casein obtained in a similar manner from raw milk dried perfectly white, and the albumin is white also."

Several samples of dark brown sweetened condensed skimmilk were examined to determine the extent of caramelization. The proteins were precipitated and the whey examined for caramel color. In every sample examined the whey failed to show any evidence of caramel color, although the proteins were very brown in color. When a solution of caramel was added to skimmilk, the whey appeared normal, for the protein had absorbed all of the caramel color. When sufficient caramel was added the proteins became saturated, thereby allowing the whey to become dark with the excess color. In spite of the similarity of this defect to caramelization, especially in color and flavor, no evidence was encountered to indicate actual caramelization of the sugars. The data presented in Table 7 naturally leads one to conclude that either the sugars do not caramelize at the temperatures and pH encountered in processing milk products, or some constituent of the milk causes a marked caramelization of the sugars. Various constituents of skimmilk were heated with sucrose and dextrose to determine their effect on caramelization. No part of the milk except proteins

TABLE 7

*The effect of heat on discoloration of sugars and proteins*

| PRODUCT | pH* | | COLOR AFTER STERILIZATION |
|---|---|---|---|
| | Control | After sterilizing | |
| 50% dextrose in water | 6.30 | 4.95 | No change |
| 25% lactose " " | 5.95 | 5.35 | " " |
| 50% sucrose " " | 6.27 | 5.93 | " " |
| Skim milk | 6.75 | 6.60 | Slight tan (normal color of sterile milk) |
| Skim + 25% lactose | 6.55 | 6.30 | Marked yellow-brown |
| " + 25% dextrose | 6.55 | 6.15 | Dark brown |
| " + 25% sucrose | 6.60 | 6.45 | Slight tan (normal color of sterile milk) |
| **Dialized albumin + 25% dextrose | 6.50 | 5.15 | Marked brown |
| **Dialized casein + 25% dextrose | 6.50 | 5.10 | Dark brown |
| Dialized casein | 6.75 | ..... | No change |
| " albumin | 6.70 | ..... | " " |

* The pH was determined by a Leeds and Northrop Potentiometer, using a quinhydrone electrode and saturated calomel half cell, before and after sterilization in an autoclave at 5 pounds pressure for 15 minutes.

** Lactose gave the same reaction.

** Sucrose failed to show any change.

were found to effect the sugars used. Albumin and casein were then heated in the presence of sugars to determine the extent of their combination. The data in table 7 clearly indicate that there is some reaction between the sugar and the protein whereby a highly colored product is formed.

*Protein Sugar Complexes.* Since there was evidently some reaction between the proteins and the sugar it seemed advisable to determine whether or not some of the constituents of the proteins would react in a similar manner. Therefore, amino acids were heated with different sugars to determine the extent of their reactions. It was interesting to find that when amino acids, especially lysine and glysine, were heated in the presence of lactose, dextrose, and levulose there was a reduction in pH accompanied by the formation of a highly colored product. The colors obtained in this manner were similar to the colors obtained by heating the sugars with milk and with milk proteins. The exact color differed with the sugar and amino acid used. Sucrose, however, failed to show any change when treated in a similar manner. The data in table 7 show that sucrose also failed to give any color with milk or milk proteins. However, under practical conditions sucrose may become inverted and cause sweetened condensed skimmilk to darken during storage.

A survey of the literature revealed that aldol and keto sugars react with amino acids readily, with the formation of highly colored products. The exact nature of the compounds is unknown, but Euler and Josephson (21) are of the opinion that the union between the sugar and amino acid is of a glucosidal nature. The reaction is probably a condensation of an amino acid with an aldehyde or ketone group of the sugar, since sugar containing neither a free aldehyde nor ketone group fail to give any such reaction. Ambler (22) states that there "is a progressive dehydrogenization of the glucose molecule during the reaction with the amino acid, with the formation of a series of compounds of progressive complexity and probably of increasing degrees of unsaturation and polymerization, properties which must accompany dehydrogenization."

There is no doubt but that the reaction in milk is between the proteins or amino acids and the sugar. Although the free amino nitrogen in milk is quite low, there is enough to carry on the reaction. Van Slyke (23) found that 5.5 per cent of the nitrogen in casein existed in a free form. This corresponds to just one-half of the lysine nitrogen, one of the two amino groups of lysine existing in a free state. Since milk as well as both albumin and casein gives a decided reaction with dextrose, it is evident that there is a large potential supply of free amino nitrogen in milk and milk products. The reaction evidently does not confine itself to the free amino nitrogen, for the entire protein is apparently changed. The casein

is dark and the color cannot be removed by dilute acid, ether, water, or alcohol. This, no doubt, explains why the whey is not colored, even though the milk is very brown. The casein apparently has marked absorptive powers. Soluble colors are immediately taken up by the casein at temperatures as low as 40° F. In one experiment the casein in skimmilk was saturated with a caramel color made from suerose. To this skimmilk 25 per cent dextrose was added. Sterilization of this sweetened skimmilk resulted in the formation of an additional dark brown color in the whey. These data confirm previous experience to the effect that the color formed from the sugar-amino acid condensation is really soluble in whey, but is absorbed by the casein.

The theory of sugar-amino condensation was further substantiated by the inhibitive action of formaldehyde. It was found that the color formation could be prevented by the presence of a small amount of formaldehyde. This was not surprising, since this chemical is well known for its affinity for amino acids. When evaporated milk was sterilized in the presence of a sufficient amount of formaldehyde, the product appeared almost as white as normal milk. This reaction alone seems sufficient to discredit the caramelization theory of brown discoloration.

### FACTORS AFFECTING THE SPEED OF THE CONDENSATION REACTION

The speed of the condensation reaction will depend on the pH, the sugar used, *i.e.*, whether or not an aldol or keto sugar is used, and the temperature.

*The Effect of pH.* Engus and Dykins (24) say that: "In agreement with observations of previous works, the effect of decreasing the hydrogen ion concentration is to increase the tendency of the sugar and amino acid to react. . . . As can be noted . . . there is little change in increasing alkalinity upon the ketone sugar-amino acid complex. A complete dissociation of the glucose glycine complex is evident after acidification of the solutions of low alkalinity, but in the solutions of higher alkalinity there appears to be either a slight destruction of glucose or the formation of some non-dissociable product."

The work of Euler and Josephson (21) also indicates that the reaction changes with a change in pH. They say that at the isoelectric point of glycine, no reaction takes place, but with an increasing alkalinity the reaction is much more complete. The explanation advanced for the increase in reaction with a greater pH is that the alkalinity favors the change of the sugar from a lactone to the free aldehyde form, which then condenses with the amino group.

The reaction in milk is also increased by the addition of alkalies. The addition of acid up to the curdling point has little effect on the appearance of the brown color. However, the addition of minute amounts of basic carbonates or phosphates have a profound effect on the development of color.

Apparently this is not due to the carbonates or phosphates themselves, but to the pH. Similar observations have been made on evaporated milk. The addition of sodium hydroxide has an identical effect. The presence of any free $\dot{O}\dot{H}$ ions causes a very dark color to appear. If the pH is much above 7, the milk may be almost black. If the milk has a pH of 7 or slightly above there may be a yellow color in the whey after heating. This is due to the presence of free OH groups which give a yellow color with all reducing sugars. There is also a possibility that at the higher pH the casein may become saturated with the soluble color compound formed as the amino acids react with the sugars. The above reaction was especially noticeable where hard water was used to dissolve corn sugar. On several occasions corn sugar sirups were badly discolored by heating with tap water. This browning caused a marked discoloration of sweetened condensed skimmilk to which the brown sirup was added. The tap water was alkaline, due to the presence of basic carbonates. When the water was acidified with hydrochloric acid or acetic acid there was no discoloration of corn sugar sirups during heating. The water should be acidified to a pH of 6.6.

*The Nature of the Sugar Used.* The speed of the reaction is related to the nature of the sugar used. The reaction depends upon the potential availability of the functional group $\diagdown C=O$ in the sugar. This group is present in both dextrose (corn sugar) and fructose in the aldehyde and ketone forms respectively. As a general rule, the aldehydes are more active than the ketones, but Neuberg and Kobel (25) state that aldol sugars react with some amino acids at a much lower rate than keto sugars.

Since corn sirup and lactose contain potential aldehyde and ketone groups they give reactions similar to that of dextrose.

The very stable linkage of the aldehyde group of the dextrose and the ketone group of the fructose in the sucrose molecule no doubt explains why sucrose fails to give condensation reactions similar to those mentioned above. However, if any of the sucrose is inverted, there may be some sugar-amino acid compound formed since sucrose yields glucose and fructose on inversion. This inversion may be due either to heat or to certain enzymes.

*The Effect of Heat.* The work of Ambler (22) and Englis and Dykins (24) shows that the coefficient of heat is very great. The data in tables 8 and 9 show that heat has a remarkable effect in producing the brown color. The length of the heating period is also very important. Milk can be exposed to relatively high temperatures for a short period with the development of less color than would be found in the same milk heated for a longer period at a lower temperature.

TABLE 8

*The effect of temperature on the time required to give a certain brown color to condensed skimmilk sweetened with corn sugar*

| TEMPERATURE OF STORAGE | TIME REQUIRED TO GIVE COMPARABLE DISCOLORATION |
|---|---|
| 100° F. | 2160 hours |
| 142° F. | 50 " |
| 200° F. | 1 " |

TABLE 9

*The effect of heat on the discoloration of sweetened condensed skimmilk made from corn sugar (standardized to 68 per cent total solids and heated as shown below)*

| TREATMENT | COLOR |
|---|---|
| Control—no heating | White to blue green |
| Heated to 180° F. 15 minutes | No noticeable effect |
| " " " " 30 " | Slight tan color |
| " " 190° F. 15 minutes | Noticeable brown |
| " " 200° F. 15 " | Quite brown |
| " " 215° F. 5 " | Dark brown |

HOW TO PREVENT THE BROWN DISCOLORATION

It was found that the color of the fresh product could be made to closely approach sucrose products in color by following either of two methods, namely: (1) avoid high forewarming temperatures, or (2) avoid forewarming the sugar with the milk.

Since the condensation reaction progresses more rapidly at higher temperatures, the brown discoloration increases as the forewarming temperature is raised. Sweetened condensed milk forewarmed to 200° F. in the presence of the corn sugar was always brown on drawing from the pan. By reducing the forewarming temperature to 150° F. the color of the finished product was made to closely approach the color of sucrose products. However, this product undergoes physical thickening at high storage temperatures. The temperature in the pan, as well as the length of the condensing period, should be reduced to a minimum in order to insure freedom from browning and physical thickening.

Since the sugar must be heated in the presence of the milk to develop the brown color, the discoloration may be prevented by adding the sugar in the form of a concentrated solution after all of the milk is out of the hot well. The sugar may be made into a 65 per cent solution and heated to 200° F. without any discoloration, provided there is no protein or milk stone in the equipment and the water is not alkaline. If there are any free $-OH$ ions in the water it should be acidified to Brome-thymol-blue (pH

6.6–6.8). The presence of milk stone will also cause a marked discoloration of the sirup during heating. The exact nature of this reaction remains to be solved, but it is probably very similar to the reactions previously discussed. During this reaction, milk stone will be partly removed from the equipment in which the sirup is heated.

If a hot well is used to heat the sirup it may be desirable to use a cover to prevent the deposition of gummy substances in the room. After all of the milk is drawn into the pan, the sugar solution should then be drawn in. By this method both the sugar and the milk may be forewarmed to any desired temperature with no danger of discoloration as far as the corn sugar is concerned. This is due to the fact that the sugar and milk mixture is exposed to a high temperature (125°–145° F.) for only a short period. However, the lactose present in milk will cause some discoloration if excessive forewarming temperatures are used.

Neither of the two methods will prevent the discoloration attendant upon storage at high temperatures. Sweetened condensed skimmilk made from corn sugar should be stored at low temperatures if discoloration is to be prevented. It is well to remember also that products containing cane sugar will darken if stored at high temperatures.

### THE IMPORTANCE OF SUGAR-AMINO COMPLEXES IN THE FOOD INDUSTRY

The existing data suggest that the color and flavor of many food products depend on the amount of sugar-amino condensation products present. The similarity of this defect to caramelization no doubt explains why the brown discoloration has always been explained on a basis of caramelization. Many canned and preserved foods are subject to brown discoloration and strong bitter flavors suggestive of caramelization. During the course of this investigation the strong "caramel" flavor and color in evaporated and sweetened condensed skimmilk was definitely traced to amino-sugar complexes.

Lintner (26) worked on the same reaction and described many flavors resulting from sugar-amino acid condensation. He reported that the aromas and flavors differed with each amino acid used. Araboni (27) also studied similar aromas, and he recovered such highly aromatic compounds as acetaldehyde, phenylacetaldehyde, and isovaleraldehyde.

There is also a possibility that this reaction may be connected with milk stone formation, since the proteins are so remarkably changed in physical characteristics. The highly colored proteins recovered from very brown milk look much like milk stone.

The sugars also exert considerable influence on the physical stability of proteins. For instance, condensed milk may be stabilized against heat by the addition of dextrose. The size of the curd particles obtained with evap-

orated, boiled, and raw milk may be related to the effect of the sugar on the stability of the protein.

## SUMMARY

(1) Corn sugar is slightly more efficient than cane sugar in preserving power.

(2) There is no difference in the types of organisms associated with condensed skimmilk sweetened with either dextrose or sucrose.

(3) Corn sugar may be substituted for one-half of the usual amount of sucrose with no danger of crystallization of dextrose.

(4) The tendency of corn sugar to cause physical thickening is probably due to the effect of the sugar on the protein. There is also a possibility that the sugar may react with the mineral salts to change the physical stability of the protein.

(5) Physical thickening may be largely avoided by forewarming the milk and sugar separately.

(6) The brown discoloration of sweetened condensed skimmilk and possibly many other food products is due to sugar protein condensation products instead of caramelization. Caramelization plays no rôle in the discoloration of dairy products.

(7) Corn sugar should be added in a concentrated solution at the end of the condensing period.

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# THE APPARENT AND TRUE SUGAR OF THE BLOOD OF LACTATING COWS FED RATIONS OF VARYING FAT CONTENT

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Studies in this laboratory by Maynard and McCay (1, 2) with lactating cows have recorded a marked lowering of the various blood lipids when most of the dietary fat was replaced by an isodynamic amount of starch. While it is generally considered that the blood sugar level tends to remain constant irrespective of the nature of the diet, there are certain conflicting reports in the literature with respect to the effect of changes in dietary fat and carbohydrate. Depisch and Hasenöhrl (3) in studying the effect of fat upon glucemic reaction reported that the administration of as much as 50 grams of olive oil had no effect upon the blood sugar curve. On the other hand, Salomonsen (4) stated that fat added to the diet raised the blood sugar of children. Rosenthal and Ziegler (5), from results of studies with rabbits and dogs, concluded that increasing the intake of raw starch produced a constant rise of blood sugar. With human beings, cooked starch produced as marked a rise in the glucemic curve as if glucose had been given, although 50 to 75 gra...s of raw starch caused no appreciable change in this curve in nine normal individuals. Schätti (6), studying the effect of the ingestion of different carbohydrates upon the blood sugar, concluded that 20 grams of starch had no effect on the blood sugar curve, but that 100 grams gave the same rise, though more delayed, as 100 grams of dextrose.

In view of these reports, it seemed worth while to take advantage of the opportunity offered by the experiments of Maynard and McCay to secure further data on this question. Further, since practically all the blood sugar values reported for cows are based upon a determination of the total reducing substances (apparent sugar) it seemed worth while to obtain some data on true sugars as well. The results of these studies are here reported.

## EXPERIMENTAL PROCEDURE

The studies were made upon the same six cows used for the blood lipid studies in the third experiment carried out by Maynard and McCay (2). Their publication gives the pertinent data relative to the animals and the

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feeding system employed. All the animals received alfalfa hay and beet pulp as roughage. The experiment was divided into three periods of five weeks each. During the first period two of the cows received a grain mixture containing approximately 7 per cent of fat. During the next five weeks they were fed the same mixture reduced to a fat content of 1 per cent by benzine extraction, the removed fat being replaced by an isodynamic amount of starch. During the final period the 7 per cent fat mixture was again fed. With the four other cows a grain mixture containing 3 per cent of fat was compared with the 7 per cent level. Two of these cows received the 7 per cent mixture during the initial and final periods and the 3 per cent mixture in the middle period. In the case of the other two cows the order was reversed. The system of feeding for each cow is indicated in the second column of table 1.

The blood samples were taken during the first, third, and fifth weeks of each period. The blood was received in a flask containing 0.2 grams of lithium oxalate per 100 cc. of blood. Samples were taken for the determinations upon the whole blood, the remainder was centrifuged for twenty minutes at 1800 R. P. M. and then the plasma was sampled. All these procedures were carried through without delay.

The method used for the determination of apparent sugar was Somogyi's modification of the Shaffer-Hartmann method (7). For the true sugar determinations, the yeast fermentation method as outlined by Somogyi (8) (9) was used. This consists essentially of analyzing the tungstic acid filtrates before and after subjecting them to the action of yeast. In this way values are obtained for the apparent sugar (total reducing substances) of the blood and the non-sugar reducing substances. The difference between the two represents the concentration of glucose or true sugar which is present in the blood. At various times during the course of the analytical work, chemically pure glucose was made up in solution with benzoic acid to check the accuracy of the methods. All determinations were run in duplicate.

## RESULTS

The results are presented in a summarized form in table 1. Each value represents an average of the three determinations made during the period in question. In order to show the trends of the individual values the data for one cow, representative of all six, are presented in full in chart 1. It is noted in both the table and chart that true sugar was determined during the last two periods only.

In general the variations in the average values from one period to another, as shown in table 1, are remarkably small in all cases. No evidence is suggested by the data that any of the shifts in dietary fat influenced either the apparent or true sugar level. There are no trends in the curves

TABLE 1

*Values for apparent and true sugar in whole blood and plasma averaged by periods.*
*(Expressed as Mg. in 100 cc. blood or plasma)*

| COW AND BREED | FAT IN GRAIN, PER CENT | APPARENT SUGAR | | TRUE SUGAR | | PROBABLE ERROR OF SINGLE DETERMINATION FOR APPARENT SUGAR | |
|---|---|---|---|---|---|---|---|
| | | Whole blood | Plasma | Whole blood | Plasma | Whole blood | Plasma |
| M | 7 | 51.3 | 49.7 | | | | |
| Jersey | 1 | 50.3 | 50.7 | 28.7 | 30.7 | | |
| | 7 | 50.3 | 55.3 | 27.3 | 35.7 | ± 2.00 | ± 3.48 |
| G | 7 | 48.3 | 51.7 | | | | |
| Holstein | 1 | 52.0 | 56.0 | 26.7 | 35.3 | | |
| | 7 | 51.0 | 52.7 | 29.3 | 33.0 | ± 1.16 | ± 1.35 |
| I | 7 | 52.7 | 48.0 | | | | |
| Jersey | 3 | 50.7 | 49.3 | 28.0 | 29.7 | | |
| | 7 | 51.3 | 54.3 | 28.0 | 34.7 | ± 1.62 | ± 3.12 |
| B | 7 | 47.0 | 52.0 | | | | |
| Holstein | 3 | 51.3 | 53.0 | 27.3 | 32.3 | | |
| | 7 | 50.3 | 53.0 | 28.0 | 33.3 | ± 1.10 | ± 2.80 |
| E | 3 | 45.7 | 49.0 | | | | |
| Holstein | 7 | 52.7 | 55.3 | 29.7 | 34.7 | | |
| | 3 | 50.7 | 53.0 | 28.7 | 33.3 | ± 2.21 | ± 1.74 |
| P | 3 | 50.5 | 49.0 | | | | |
| Guernsey | 7 | 51.3 | 54.0 | 29.0 | 34.0 | | |
| | 3 | 52.7 | 57.7 | 29.7 | 38.0 | ± 1.58 | ± 1.97 |
| Range of individual values | | 45–57 | 43–60 | 26–32 | 24–40 | | |
| Mean of all determinations | | 51.22 ± .26 | 53.69 ± .41 | 28.31 ± .22 | 33.72 ± .40 | ± 1.54 | ± 2.46 |

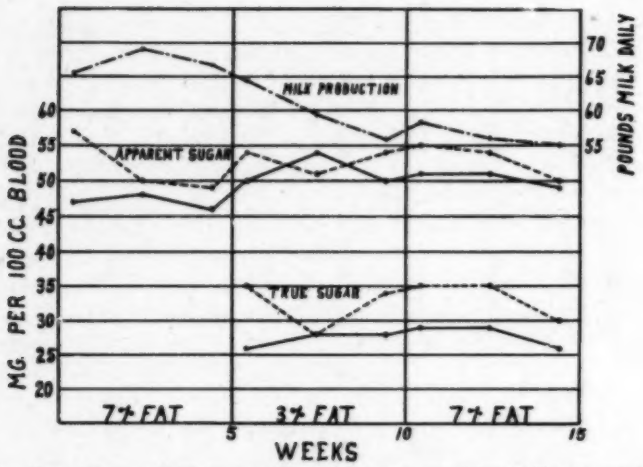


CHART 1.—THE APPARENT SUGAR AND TRUE SUGAR IN THE WHOLE BLOOD AND PLASMA, AND THE MILK PRODUCTION OF COW B.

——— Whole Blood - - - - Plasma —·— Milk Production

based on the individual values, as presented in chart 1, which indicate any such relationship. Neither were any relationships found by plotting similar curves for the other cows. A thorough statistical study of all the individual values revealed no relationship between the fat or carbohydrate intake and blood sugar level. This finding stands in marked contrast to the changes in the lipids in these same blood samples as shown in charts 16, 17 and 18, of the publication of Maynard and McCay (2).

It is interesting to note in table 1 the uniformity of the values among the different individuals, particularly as three different breeds are represented. The ranges of the single determinations are shown in the next to the last line. The mean values show a very small probable error. The probable errors for the single determinations were also found to be small, as is shown in the last two columns for the apparent sugar values.

It is shown in table 1 that only approximately 60 per cent of the total reducing substances is true sugar. It is seen that the values for plasma are somewhat higher than for whole blood. This true sugar represents the difference between the values for apparent sugar and reducing non-sugar. The results for reducing non-sugar are in close agreement with the few data reported by West, Scharles and Peterson (10) for beef blood. Their values ranged from 22 to 25 mg., expressed as glucose. Our reducing non-sugar values in both whole blood and plasma compare quite favorably with similar data which have been reported for human blood.

We have been interested to compare our data with those reported for lactating cows by other investigators. In table 2, we have assembled various values which appear in the literature. So far as can be determined from the methods used, all of these values represent total reducing substances. The wide ranges reported by most of these investigators suggest that blood sugar is a highly variable figure in lactating cows. Our corresponding values, apparent sugar in the whole blood, show a much narrower range than most of those cited in the table, despite the changes in the ration fed. Whether the wide ranges listed in table 2 represent true physiological differences or merely variations in chemical procedure is a question that cannot be answered.

Numerous European investigators, notably Widmark and Carlens (11), and some in America (12) (13), have observed that a hypoglucemic condi-

TABLE 2

*Some blood sugar values on lactating cows as reported by various investigators*

| AUTHOR | METHOD | MG. GLUCOSE PER 100 CC. BLOOD |
|---|---|---|
| Anderson, Gayley and Pratt | Folin-Wu | 43.2– 68.4 |
| Auger | G. Bertrand | 55 – 75 |
| Awdejewa, Prowatorowa, Sawitch and Thal | Hagedorn-Jensen | 33 – 68 |
| Carlens and Kristownikoff | Bang | 63 – 92 |
| Cary and Meigs | Folin-Wu (revised) | 54.7– 73.6 |
| Hayden | Folin-Wu | 45.6– 52.2 |
| Hayden and Fish | Benedict | 46 – 52 |
| Hayden and Sholl | Folin (colorimetric) | 37.2– 97.6 |
| Hayden and Tubangi | Benedict | 77 –120 |
| Hewitt | Folin-Wu | 50.6– 63.5 |
| Kaufmann and Magne | Cl. Bernard and Bierry and Portier | 49 – 93 |
| Little, Keith and Fawns | Hagedorn-Jensen | 56 –104 |
| Moussu | Fontes and Thivolle | 47.5– 79.5 |
| Norris and Chamberlain | McLear | 49 –105 |
| Porcher | Bierry and Portier | 38 – 40 |
| Richter | Hagedorn-Jensen | 55.7– 60.8 |
| Riddell | Folin (Micro) | 35 – 85 |
| Scheicher | Hagedorn-Jensen | 54.3– 83.6 |
| Scheunert and Pelchrisim | Bang | 62.5–100 |
| Schlotthauer | Folin | 52.6– 68.45 |
| Shope | Folin | 38 – 48 |
| Schwarz | Hagedorn-Jensen | 44 –121 |
| Schwarz and Mezler-Andelberg | Hagedorn-Jensen | 60 – 78 |
| Turner and Herman | Shaffer-Hartman | 52 |
| Volker | Hagedorn-Jensen | 47 – 91 |
| Widmark | Bang, Hagedorn-Jensen | 53 – 76 |
| Widmark and Carlens | Bang | 40 – 88 |

tion exists in heavily milking cows. Since there were marked changes in milk yield in the course of our experiments, as is shown for one cow in chart 1, a statistical study was made of the relationship between apparent sugar and milk yield. The correlation coefficient was found to be –.2399, which in itself is not great enough to be significant but which does indicate a slight tendency toward lower blood sugar with higher milk production. Auger (14) and Schwarz and Mezler (15) have concluded that the blood sugar values rise markedly with advancing lactation, a conclusion which is not supported by the findings of Scheicher (16) that the values tended to be highest shortly after calving. Our data, covering only a portion of the lactation period, tend to support the conclusion of Riddell (17) that advancement of lactation has little effect upon blood sugar level.

## SUMMARY

In experiments with six milking cows the substitution of part or approximately all of the fat of the grain mixture by an isodynamic amount of starch was without effect upon either the apparent or true sugar in the whole blood or plasma. The mean values for apparent sugar were 51.22 ± .26 mg. per 100 cc. whole blood and 53.60 ± .41 mg. per 100 cc. plasma. The mean values for true sugar were 28 ± .22 for whole blood and 33.72 ± .40 for plasma. The correlation coefficient between apparent blood sugar and milk yield was –.2399.

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# SUBSTANCES ADSORBED ON THE FAT GLOBULES IN CREAM AND THEIR RELATION TO CHURNING. II. THE ISOLATION AND IDENTIFICATION OF ADSORBED SUBSTANCES*

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In a recent paper (1), the authors show that the fat emulsion in cow's milk is stabilized by a layer of adsorbed material which is so firmly held at the surface of the fat that rich cream can be repeatedly diluted with distilled water and reseparated by means of a centrifugal cream separator without breaking the emulsion. The present paper is concerned with the isolation and identification of this material from the surface of milk fat globules in the washed cream.

In general, the method used for the isolation of the adsorbed material consisted in churning cream that had been washed six to eight times with distilled water, using four volumes of water at each washing. The fat stabilizing material was recovered from the buttermilk, butter washings and melted butter. The butter was melted below 60°C., the melted butterfat poured off and the non-fat fraction either supercentrifuged and added to the centrifuged buttermilk or extracted in a separatory funnel with ethyl alcohol and ether. The details of the washing and churning experiments have been described in the previous paper (1).

This method of isolation assumes that the solids-not-fat of the washed cream constitute the material most closely adsorbed on the surface of the fat in the original cream. The procedure has several advantages. By using heavy cream as the starting material, the solids-not-fat in the cream are reduced to a new minimum after each dilution and reseparation. This greatly facilitates the removal of milk solids from the fat without destroying the emulsion. When properly carried out, the establishment of a new equilibrium between the material on the surface of the fat and the dispersions medium at each dilution, does not materially reduce the stability of the oil-in-water emulsion. Although the stabilizing material that remains concentrated at the fat-water interface may not be the only emulsion stabilizing material present in the original cream, it is reasonable to suppose that it is the most important.

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By churning the washed cream, large quantities of butterfat are removed more easily than by extraction. By avoiding the use of fat solvents at this stage of the procedure the recovery of any fat-soluble stabilizing substances is assured. All previous workers have found protein in the fat-stabilizing material isolated from milk and many investigators have also reported inorganic constituents associated with the protein. In the procedure adopted in this investigation, a study of the constituents of the "membrane" as well as the physical properties of the "membrane" material can be made on the supercentrifuged buttermilk.

### INORGANIC CONSTITUENTS

After churning the washed cream, the supercentrifuged buttermilk was concentrated at reduced pressure using a temperature below 50°C. The concentrated buttermilk remained a uniform, milky liquid. No precipitation occurred during concentration. It was dialyzed in a collodion[1] or cellophane bag against running distilled water for 42–92 hours. Toluene was added as a preservative. Determinations of the total solids, ash, calcium and phosphorus were made before and after dialysis. Calcium was determined in the ashed samples by the method recommended by Clark and Collip (2) for blood calcium. This includes a precipitation of the calcium with ammonium oxalate followed by titration with .01 N potassium permanganate. The Briggs (3) modification of the Bell-Doisy method for blood phosphorus was used. The results of the analyses are given in table 1.

The dialysis experiments show that the fat stabilizing material can be obtained free from calcium. If the protein present occurs as a calcium compound the calcium is evidently readily and completely dissociated inasmuch as the small amount of calcium found in some of the concentrates was with one exception practically all removed by dialysis with distilled water. The ash gave qualitative tests for sodium and potassium but the amount of these bases was not sufficient to bind all the phosphorus present. Samples ashed directly without the addition of another base yielded residues insoluble in dilute hydrochloric acid. A measured volume of calcium acetate was therefore added before ashing. The ash from the calcium acetate solution employed gave tests for sodium and potassium but was free from phosphorus. All the ash samples gave negative tests for magnesium. The higher percentage of ash in sample 3 after dialysis in comparison with sample 2 cannot be explained except on the basis of relatively different proportions of protein and materials containing non-dialyzable phosphorus. The ash of all the protein samples contained phosphorus and sulfur as will

[1] The collodion bags were hardened by immersion in 1 per cent gelatin solution, followed by immersion in 2 per cent formaldehyde solution. The bags were then washed thoroughly before using.

TABLE 1

*Ash, calcium, and phosphorus in dry "membrane" material*

| SAMPLE | COMPONENTS OF CONCENTRATE | TIME OF DIALYSIS | BEFORE DIALYSIS AGAINST DISTILLED WATER | | | AFTER DIALYSIS AGAINST DISTILLED WATER | | |
|---|---|---|---|---|---|---|---|---|
| | | | Ash | Calcium | Phosphorus | Ash | Calcium | Phosphorus |
| | | *hrs.* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 1 | Buttermilk<br>Butter washing<br>Butter S. N. F.<br>High M. P. glycerides | 42 | 2.24 | 0.25 | 0.61 | 1.54 | 0.06 | 0.43 |
| 2 | Buttermilk<br>Butter washing<br>Butter S. N. F. | 45 | 2.56 | 0.19 | 0.87 | 2.19 | 0.15 | 0.71 |
| 3 | Buttermilk<br>Butter washing<br>Butter S. N. F. | 88 | 2.63 | 0.03 | 0.78 | 2.34 | 0.00 | 0.69 |
| 4 | Buttermilk<br>Butter washing | 92 | 2.43 | 0.00 | 0.83 | 2.46 | 0.00 | 0.80 |

be shown later. The lower percentage of ash in sample 1 after dialysis may be attributed to the relatively high content of high melting point glycerides. By far the major part of the phosphorus in the concentrates remained non-dialyzable against distilled water. The absence of calcium and magnesium in the residues eliminated the possibility of insoluble inorganic phosphates.

Our dialysis experiments indicate that the calcium found in the "membrane" material isolated by other workers was an impurity and that the major part of the phosphorus which they either overlooked or considered an impurity, remains non-dialyzable, occurs in organic combination and definitely constitutes a part of the material adsorbed on the surface of the fat globules.

### PROTEIN FRACTION

*Chemical composition.*—The protein fraction in the buttermilk concentrates was purified for chemical study as follows. After dialysis against running distilled water, as previously described, the protein material was precipitated with dilute HCl solution at pH 3.9–4.0. The precipitated concentrate was then dialyzed in a heavy cellophane bag against distilled water acidified to pH 3.9–4.0. The precipitate was then filtered off, washed

with acetone to remove traces of adhering butterfat, and extracted in a Soxhlet with hot purified ethyl alcohol, followed by alternate extraction with hot absolute ethyl alcohol and anhydrous ethyl ether. The extractions were continued until the ether extract yielded no residue on evaporation or until the phosphorus content of the protein remained constant. The alcoholic extracts were saved for the phospholipide study.

After extraction, the protein was dried in a desiccator over $H_2SO_4$, ground, put through a 100 mesh sieve and analyzed for total phosphorus, sulfur, and nitrogen. Phosphorus was determined in the ashed sample according to the Briggs (3) procedure. Sulfur was determined by the procedure of Hoffman and Gortner (4). The sample was dispersed in hydrochloric acid, oxidized with Denis-Benedict's reagent and the sulfur precipitated as $BaSO_4$. Nitrogen was determined by the Kjeldahl method. Table 2 gives a summary of the results of these analyses in comparison with

TABLE 2

*Average composition of "membrane" protein in comparison with previous preparations, haptein and the known proteins of milk*

| PROTEIN | PHOSPHORUS | SULFUR | NITROGEN |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| Membrane protein— | | | |
| Preparation 1 | 0.27 | 0.96 | 11.84* |
| Preparation 2 | 0.37 | 0.96 | 12.11 |
| Preparation 3 | 0.30 | 0.94 | 12.64 |
| Palmer and Samuelsson's membrane protein (6)** | 0.38 | 0.73 | 10.92 |
| Haptein (5) | ..... | 2.58 | 12.05 |
| Casein (7, 8) | 0.82 | 0.82 | 15.65 |
| Lactalbumin (9) | trace | 1.92 | 15.43 |
| Globulin (9) | 0.24 | 0.86 | 15.44 |
| Alcohol soluble protein (8) | 0.08 | 0.95 | 15.71 |

* Value for nitrogen low. Extraction of protein was continued until there was no reduction in the phosphorus content but later material soluble in hot 60 per cent ethyl alcohol was found.

** Sample of membrane protein prepared in this laboratory in 1923.

those recorded in the literature for casein, lactalbumin, globulin, the alcohol soluble protein of milk and Hattori's (5) haptein. A sample of membrane protein prepared in this laboratory from washed cream by Palmer and Samuelsson (6) was analyzed by us and the data are included in table 2 for comparison.

Haptein, as reported by Hattori, Palmer and Samuelson's protein from washed cream and our protein from washed cream are each characterized by a low pecentage of total nitrogen. However, haptein and the washed cream proteins differ greatly in sulfur content. The ash of haptein is

stated to contain calcium and phosphorus but the percentage of neither is reported. None of the samples of protein that were prepared from washed cream in the present study contained calcium. The phosphorus content of our preparations averaged 0.31 per cent. The percentage of phosphorus, sulfur, and nitrogen indicates that the protein is not casein, lactalbumin, globulin or the alcohol soluble protein of milk.

*Properties of the protein.*—Character of aqueous solution.—When the buttermilk, butter washings and butter plasma were supercentrifuged (speed 40,000 r.p.m.) a layer of bluish gray protein collected on the inside of the bowl of the Sharples supercentrifuge. This was carefully removed and used for a study of the physical properties of the protein, inasmuch as the concentrate was subjected to long dialysis followed by extraction with solvents to remove lipide substances, procedures which denature proteins and greatly alter their physical properties.

The fresh protein removed from the bowl of the supercentrifuge redispersed in distilled water sufficiently well to withstand ordinary centrifuging. An ultramicroscopic examination of the aqueous dispersions showed unclustered protein particles and many small clusters of the same particles. Another sample of protein obtained by direct extraction of the material from the melted butter after drying gave a fine powder which readily imbibed water yielding a grayish colored sol stable to ordinary centrifuging. The ultramicroscopic examination of this sol also showed both unclustered and small clustered particles. The dispersion was clearly colloidal, although slightly coagulated. The "visibility" of the particles suggests a type of colloid resembling casein as it exists in milk or when dispersed in water by means of calcium. The "membrane" sol, however, contained no calcium.

*Isoelectric point.*—Equal volumes of buffer solutions having pH 4.54, pH 4.36 and pH 4.09 were added to the above protein dispersions and also to samples of supercentrifuged buttermilk and supercentrifuged butter plasma. These solutions were then examined with the ultra microscope. The results of these examinations are shown in table 3.

The ultramicroscopic examinations confirmed preliminary tests which showed that precipitation of material began at pH 4.6 and that a clear serum separated at pH 3.9–4.0. A redispersed extracted protein (which had not been dialyzed) from melted butter did not whey off even at pH 3.9 although it could be filtered clear. This protein was no doubt partially denatured. In contrast with the above results, the protein from a redispersed skim milk powder when examined in the ultramicroscope, was clustered and completely precipitated at pH 4.54.

Since the protein material used for the determination of the isoelectric point contained phospholipides, the question arises as to what extent their presence influenced the value obtained. Mayer and Terroine (10), Fein-

TABLE 3

*Ultramicroscopic examinations*

| | ORIGINAL SOL | pH 4.54 | pH 4.36 | pH 4.09 |
|---|---|---|---|---|
| Bowl protein | Unclustered protein particles<br>Small clusters | No evident difference from original sol | Increase in size and number of clusters<br>Precipitation not complete | Precipitation on standing<br>No dispersed protein in serum<br>pH of serum 3.95 |
| Crudely extracted protein | Unclustered protein particles<br>Small clusters | Unclustered protein particles present | Increase in size and number of clusters | Does not precipitate completely on standing<br>Small clusters and unclustered protein present<br>pH 4.12 |
| Supercentrifuged buttermilk | Mostly unclustered particles<br>Few clusters<br>Little fat | Noticeable clustering<br>Small clusters | | Precipitation complete after standing but not instantaneous |
| Supercentrifuged material from melted butter | Great deal of fat<br>Clustered material adsorbed on fat globules giving a lace-work effect | Little effect noted | | |

schmidt (11), and Parsons (12) in studying the precipitation of lipide-protein complexes found the acidity at which maximum precipitation occurs to be between the isoelectric point of the separate constituents. The isoelectric point of milk lecithin has not been determined but the isoelectric range for other lecithins lies between pH 2.2–6.3 (11, 13, 14, 15). If the non-dialyzable phosphorus in the concentrate is due to the presence of phospholipide-like substances, they would be precipitated with the protein at pH 3.9–4.0. The hydrogen-ion concentration (pH 3.9–4.0) at which most complete precipitation of the bowl protein and buttermilk takes place is therefore the isoelectric point of the protein-phospholipide complex rather than the isoelectric point of the protein alone. The fact that there appears to be no difference in the pH at which the bowl protein is completely precipitated and the pH at which material in the buttermilk is completely precipitated seems to indicate that the phospholipide is very intimately associated with the protein. Failure to obtain phosphorus-free protein may be due to some adsorbed phospholipides.

Several publications have appeared in the past few years on the isoelectric point of the fat globules in milk. Mommsen (16), Prieger (17) and Mohr and Brockmann (18) using the microscopic cataphoresis cell of Michaelis have attempted to determine the "umladungs" point of milk fat emulsion in water alone and of the natural milk fat emulsion in milk or cream or when stabilized by a known protein such as casein. The same

procedure was employed in each of the studies. The material was diluted with buffers of known pH value until a value was obtained at which the fat globules showed no greater tendency to migrate to one electrode than the other when e.m.f. was applied.

Mommsen obtained a value of pH 4.11 for milk diluted 1:10 and a value of pH 4.17 for cream diluted 1:100. He also observed that NaCl lowered the pH value and $CaCl_2$ raised it for the cream, depending on the concentration of the cations, while gelatin shifted it to the isoelectric point of gelatin. Mommsen suggested that the latter finding indicated that Hattori's haptein may have the isoelectric point which he had observed for the fat globules of cream.

Prieger obtained values of pH 2.27–3.07 for butterfat in water alone in contrast to a value of 3.95 for a butter emulsion containing one per cent fat and a value of 4.21 for milk. She attributed the higher isoelectric point of the fat in the butter emulsion to the presence in butter of the protein "membrane" carried over from the milk. She did not attempt to isolate the protein.

The isoelectric points found by Mohr and Brockmann were 2.96 for milk fat in water, 4.24–4.30 for cream, 4.34 for milk and 4.44 for fat emulsion containing added casein. Seasonal variations or different nutritive conditions are suggested by Mohr and Brockmann for the somewhat higher values obtained by them in comparison with Prieger's results.

Nugent (19) has determined the isoelectric point of the fat globules in cream to be pH 4.1 in a study of cream diluted with various buffers using a Northrop-Kunitz microcataphoresis cell. This value corresponds more nearly to that observed by Mommsen.

The isoelectric point values reported which are higher than we observed for the isolated membrane material and for the butter emulsion observed by Prieger are no doubt explained either by the presence of divalent cations or to the presence of other milk proteins in the outer shell of interfacial material.

*Stability toward heat, rennin, acid, base and salts.*—The protein was not coagulated by heat alone or by rennin at 40°C., even in the presence of calcium chloride. On addition of dilute hydrochloric acid, precipitation began near pH 4.6 but was most complete at pH 3.9–4.0. The precipitate could be readily redispersed in an excess of HCl or dilute NaOH (pH 5–6) or could be reprecipitated from its acid or alkaline solution in the isoelectric range.

The salt effects on the material in the supercentrifuged buttermilk, both in the cold and on heating are given in table 4. The most striking effect was the extreme sensitivity of the sol to Ca ions on heating.

The possibility of the "membrane" protein being casein or lactalbumin can be eliminated because of its failure to coagulate with rennin or coagulate

TABLE 4

*The effects of salts on the "membrane" sol*

| SALT | CONCENTRATION | COLD | HEATED TO BOILING |
|---|---|---|---|
| NaCl | Few crystals | No effect | No effect |
| | 30 per cent | Very fine precipitate | |
| $Na_2SO_4$ | Few crystals | No effect | No effect |
| $(NH_4)_2SO_4$ | Few crystals | No effect | No effect |
| | ⅛ saturation | Precipitate formed | |
| | ¼ " | " " | |
| | ½ " | Precipitation most complete | |
| $CaCl_2$ | Few crystals | No effect | Complete precipitation |
| $MgSO_4$ | Few crystals | No effect | Precipitation |
| | Saturation | Precipitation | |

on heating alone or in the presence of monovalent cations. Casein is coagulated by rennin at 40°C., even in the presence of lactalbumin and globulin. In whole milk, heat-coagulated-lactalbumin and globulin do not precipitate in the presence of the casein but since casein was not present here, the failure of the protein to precipitate on heating even in the presence of monovalent cations could not be attributed to this cause.

Globulin if present would be expected to precipitate when the concentrates were dialyzed against running distilled water for 88–92 hours. All of the dialyzed concentrates were well dispersed sols, no precipitation taking place during dialysis.

The physical and chemical properties of the aqueous solution of the "membrane" material support the results of the general chemical composition in showing that the protein is not casein, lactalbumin or globulin.

### PHOSPHOLIPIDE FRACTION

Three phospholipides, lecithin, cephalin and a diaminophospholipide have been identified in cow's milk (20, 21, 22). Because of the large percentage of non-dialyzable phosphorus in the concentrate from the buttermilk, butter washings and melted butter, the hot alcoholic extracts from the precipitated concentrates were examined for organic phosphorus-containing compounds. A procedure was adopted which would permit the detection of lecithin, cephalin and sphingomyelin. The essential features of the procedure are shown diagrammatically in figure 1. Chemical data obtained on certain fractions in comparison with known phospholipides are given in table 5. Phosphorus was determined by the Briggs (3) method

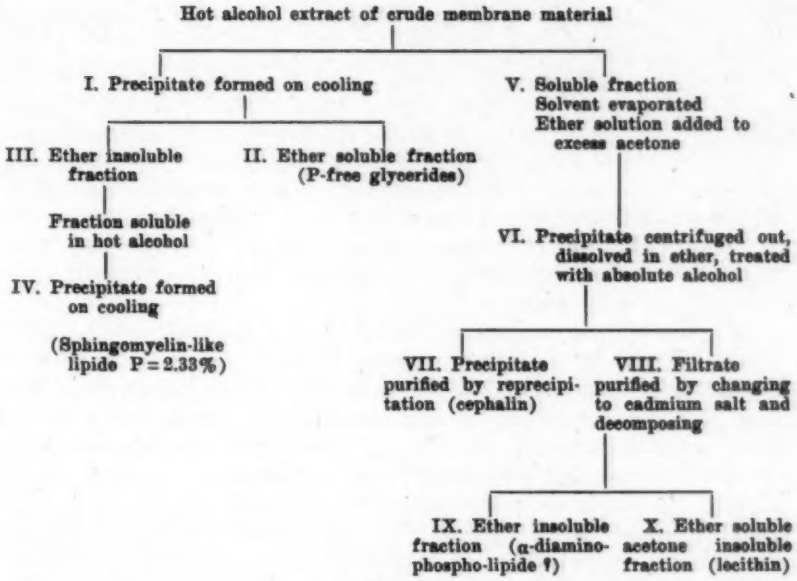


FIG. 1

TABLE 5

*Phosphorus and nitrogen content of phospholipides*

| | PHOSPHORUS | NITROGEN | P : N RATIO |
|---|---|---|---|
| | *per cent.* | *per cent* | |
| Lecithin found | 2.70 | 1.75 | 1: 1.4 |
| | 3.85 | | |
| Milk lecithin[1] | 3.30 | 2.56 | 1: 1.5 |
| Butter lecithin[1] | 2.85 | 2.29 | 1: 1.7 |
| Calculated for oleyl-stearyl lecithin[1] | 3.85 | 1.73 | 1: 1.0 |
| Oleyl-palmityl lecithin[1] | 3.99 | 1.80 | 1: 1.0 |
| Cephalin found | 3.29 | 2.39 | 1: 1.6 |
| | 2.58 | | |
| Milk cephalin[1] | 2.84 | 1.56 | 1: 1.2 |
| Brain cephalin[2] | 3.58 | 1.80 | 1: 1.1 |
| Ether insoluble phospholipide found | 3.71 | 4.85 | 1: 2.9 |
| Milk phospholipide[3] | 2.59 | 2.45 | 1: 2.1 |
| Brain sphingomyelin[4] | 3.60 | 3.40 | 1: 2.1 |

[1] Bischoff (22).
[2] Levene and Rolf (23).
[3] Osborne and Wakeman (19).
[4] Levene (24).

for blood phosphorus. The Koch-McMeekin (25) direct nesslerization method for nitrogen was used.

Precipitate I was copious and white in color. Most of it was somewhat soluble in ether, 100 cc. dissolving 0.10–0.36 grams. The ether soluble fraction II yielded crystals having the appearance of sphingomyelin, but these proved to be a mixture of phosphorus-free glycerides of high melting, saturated fatty acids. Different crops of crystals showed melting points of 53.6–58.4°C., solidifying points of 43.2–46°C., and saponification values of 194.2–197.2. Most of this material was obtained from the crude membrane recovered from the buttermilk secured on melting the butter churned from the washed cream.

Fraction III was obtained by exhaustive ether extraction of Precipitate I in a Soxhlet. It was a gray, gummy material. After purification by crystallization from alcohol, it contained 2.33 per cent phosphorus. MacLean (26) reported 3.8–4.2 per cent phosphorus in sphingomyelin but the diaminophospholipide isolated from milk by Osborne and Wakeman (21) contained 2.59 per cent phosphorus. The evidence at hand is insufficient to identify the substance as sphingomyelin, although like this lipide it is soluble in alcohol and insoluble in ether. Insufficient material was obtained for nitrogen determination. Sphingomyelin in milk has not been extensively investigated, the only results reported being those by Brodrick-Pittard (27) and Osborne and Wakeman (19). Differences in solubility of the products obtained by Brodrick-Pittard was the basis for the assumption that milk contained two phospholipides. Osborne and Wakeman (19) also reported the presence of a monoamino- and a diaminophospholipide in milk differing in solubility in acetone and ether. Studies of the hydrolysis products have not been made.

Cephalin and lecithin were separated from the alcohol soluble fraction V, according to the procedure shown in figure 1. The lecithin was purified first through the cadmium salt and then by reprecipitating it twice from ether by acetone. During these purifications, part of the material originally soluble in ether became insoluble. The total amount of material recovered in this way was small in comparison with the lecithin. The supposition is that it represented either partially decomposed lecithin or cephalin or a diaminophospholipide that was originally soluble in ether because of the presence of lecithin and cephalin.

Although the analyses of the various phospholipide fractions reported in table I show lower phosphorus and higher nitrogen contents in comparison with the analyses of phospholipides from other sources the presence of phospholipide substances is apparent. The small quantity of these substances obtained prevented further purification. Differences in solubility and the P : N ratio indicate the presence of three phospholipides, lecithin, cephalin and a diaminophospholipide.

*Proportion of phospholipides in membrane material.*—Calculated on the basis of the percentage of non-dialyzable phosphorus in the dry matter of the original crude membrane material, the percentage of phospholipides with an average content of 3.85 per cent phosphorus varied between 17.5 and 20.4 per cent of the different membrane preparations. The data are shown in table 6. These data are subject to the error due to the presence

TABLE 6

*Calculation of proportion of phospholipides in dry crude "membrane" material*

| TOTAL SOLIDS IN CONCENTRATE AFTER DIALYSIS | NON-DIALYZABLE PHOSPHORUS IN TOTAL SOLIDS | PHOSPHOLIPIDES[1] IN TOTAL SOLIDS | PHOSPHOLIPIDES |
|---|---|---|---|
| *grams* | *grams* | *grams* | *per cent* |
| 15.90 | 0.1073 | 2.78 | 17.5 |
| 32.85 | 0.2245 | 5.83 | 17.7 |
| 16.27 | 0.1278 | 3.32 | 20.4 |

[1] Calculated on the basis of phospholipides containing 3.85 per cent phosphorus.

of the high melting glycerides. It is not possible to give a figure for the percentage of these glycerides present, but there is every reason to believe that there was insufficient to increase the relative proportion of phospholipides to the point where they would be regarded as constituting the major portion of the active "membrane" material. On the contrary the present study points to protein constituting the major part of the material isolated from washed cream.

## AMOUNT OF "MEMBRANE" MATERIAL IN MILK

The mechanical difficulties involved in the isolation, separation and purification of the material adsorbed on the surface of the fat globules do not warrant a calculation of the amount present. Van Dam and Sirkes (28) calculated concentrations of adsorbed protein between 0.005 and 0.245 per cent of the milk based on the differences between observed percentages of protein in the fat-free portions of creams and the fat-free skimmilks from which the creams were obtained. Hattori (29) and Hattori and Ogimura (30) have reported recoveries of 0.01, 0.02 and 0.028 per cent haptein from milk. The fat content of the milk was not stated. An estimation of the recovery of crude material from washed cream, based on the total solids remaining from the supercentrifuged and dialyzed buttermilk, butter washing, butter serum and protein recovered from the centrifuge bowl, compares favorably with the higher percentages found by these investigators. However, the figures merely indicate that small amounts of the active stabilizer are recovered. The total solids include very small fat globules and the high melting point glycerides which pass through the

supercentrifuge. On the other hand, phospholipides remaining dissolved in the butterfat are not recovered. The calculated values for a milk containing 3.5 per cent butterfat are given in table 7.

TABLE 7

*Recovery of crude membrane from 3.5 per cent milk*

| WASHING EXPERIMENT | TOTAL SOLIDS AFTER DIALYSIS | BUTTERFAT IN WASHED CREAM | RECOVERY FOR 3.5% MILK |
|---|---|---|---|
| | *grams* | *grams* | *per cent* |
| 2 | 15.90 | 1779 | 0.031 |
| 3 | 40.03 | 4793 | 0.029 |
| 4 | 44.05 | 6510 | 0.023 |

The figures show a smaller percentage recovery when only the buttermilk and butter washings are supercentrifuged.

## DISCUSSION

The experimental findings show that the material adsorbed on the surface of fat globules is composed of a mixture of phospholipides[2] and proteins. The fact that the phospholipides have been overlooked by all previous investigators with the exception of Palmer and Samuelsson (6) can perhaps be attributed to the methods of isolation employed. The fat stabilizing substance was recovered by other workers from fat globules after an extraction of the butterfat with solvents, a procedure which also removes the phospholipides. Whether or not these phospholipides are identical with those found dispersed in milk and reported in the literature cannot be stated. The extremely small quantities found make such a comparison impossible. With regard to the protein, however, the method of isolation and its composition and properties together indicate that it is not identical with any of the other known milk proteins or a mixture of these proteins.

[2] The literature contains several references which imply that Dornic and Daire (37) first demonstrated the presence of lecithin on the fat globule surfaces in cow's milk. It is true that these workers advanced the hypothesis that lecithin forms a part of Storch's Stimmenmembran, but they offered no evidence whatever to support the hypothesis other than the fact that cream and buttermilk are richer in lecithin than the original whole milk. Other substances, *e.g.*, reductase and amylase, are likewise known to concentrate in the cream and buttermilk but this fact alone does not constitute evidence that these enzymes form a part of the surfaces of the fat globules. The entire reference of Dornic and Daire to this problem is confined to the following words: "Il est très probable que les lecithines font partie de la fameuse Stimmenmembran de Storch, non pas que nous considérions cette membrane comme nettement de finie mais en admettant avec Lindet qu'elle es constituee par une enveloppe de matiere agotée qui serait fixée sur de globule par moléculai attraction. Pendant le barrattage qui amène la soudure des globules gras, cette gangue se désagrège et se retrouve par la suite dans le babeurre."

The possibiliy of the membrane protein being one of the components of a casein system is also excluded by its low nitrogen content. Each of the seven fractions of casein prepared by Linderstrøm-Lang (31) contained the same percentage of nitrogen as the casein from which the fractions were isolated.

The protein and mixture of phospholipides may not always occur in the same ratio. Inasmuch as other milk constituents vary in amount with different animals, breed, stage of the laction period, time of milking, time between milkings and season of the year, it would seem reasonable to expect a variation in the proportion of the components of the fat stabilizer.

The ratio of these components together with equilibrium conditions at the fat-plasma interface may account at least in part for differences in the stability of the fat of various milks. Great differences in the churnability of creams in industry have been noted. That the nature of the material at the surface of the fat in artificial emulsions plays an important part in the churnability of these emulsions is shown by the churning experiments presented in our previous paper (1). By analogy, then, any difference in the ratio of the components of the fat globule stabilizer in cow's milk would affect the churnability of the cream from this milk. Moreover, equilibrium changes in the dispersions medium (milk plasma), such as the addition of hydrogen ions or changes in other ionic concentrations, must alter the surface activity of the adsorbed fat stabilizer. Although the mechanical operations of the churning process are essential for the formation of butter, the electrical and equilibrium conditions at the fat-plasma interface cannot be ignored.

The experiments conducted in this investigation were not planned primarily to prove or disprove any theory of churning. Nevertheless, some of the experimental findings show that the foam theory of churning advanced by Rahn (32) not only requires considerable modification but that an inversion of phases does take place during some stage of the butter making process. For example, surface active substances, protein and phospholipides, are recovered in large proportions in the free buttermilk when churning cream that has been washed practically free of all water "soluble" material except its actual emulsion stabilizing fraction,—the fat "hulls." One phase of the foam theory of churning assumes that the coagulated gel-like protein hulls around the fat globules constitute the true continuous phase of butter. If this were true, then cream which has been washed free of all protein except that found in the "hull" material should not yield any protein or other colloidal materal in the free buttermilk when such cream is churned. The fact that the free buttermilk, the butter washings and also the melted butter in such a churning contain protein and phospholipides not detectable in the cream washings does not support the idea of a continuous aqueous phase in butter surrounding all the fat globules

originally present in the cream and composed of the original membranes that had stabilized the cream emulsion.

These facts make it necessary to modify all theories of churning that assume a super cream structure for butter resulting from some alteration in the surface active properties of the original fat membranes which causes the fat globules to stick together. The actual liberation of a large part of the surface active material from the fat globules during churning can no longer be denied. Mohr and Brockmann (33) (34) have presented indirect evidence for this fact by showing that the surface tension of dairy products decreases in the order skim milk > whole milk > cream and that further decreases are noted when comparing the surface tension of pasteurized sweet cream during churning, buttermilk having the lowest surface tension. Although acid cream and aged cream do not show this decrease in surface tension during churning Mohr and Brockmann interpret their data as follows: whole milk and cream have a lower surface tension than skimmilk because of the greater amount of surface active material present (on the surface of the fat globules). This surface active material is released during churning thus causing still further reduction in surface tension. Regardless of whether the surface tension data are reliable, our experiments with washed cream are a direct demonstration of the fact that much colloidal material is removed from the fat globule surface during churning.

The low isoelectric point of the fat membrane material in comparison with the other proteins of milk undoubtedly has a significance for the practical churning operation. The cataphoresis experiments of Mohr and Brockmann (18) were for the purpose of determining the relation between the isoelectric point of the fat globules and the exhaustiveness of churning. In general their data point to the lowest fat losses in the buttermilk when cream is churned at pH 4.20–4.30. Guthrie and Sharp (34) have found that the time required for churning natural cream exhibits one of its minima at about pH 4.2 where the range of pH 0.5–13.5 is studied. Cream washed twice with 9 volumes of water, however, exhibited a marked maximum churning time at pH 4.0. Fat losses were not reported in their study but the observations of lowest fat losses obtained by Mohr and Brockmann, and the observations of minimum churning time obtained by Guthrie and Sharp, at the same pH do not correspond with the practical observation that lowest fat losses are usually associated with longer rather than more rapid churning. The meager data at hand, therefore, indicate that the exact relation of the isoelectric point of the fat membrane material to various dairy manufacturing operations remains to be determined.

No accurate data are available as to the distribution of the membrane material of the original cream between the free buttermilk and washings, the microscopic water phase of the butter, and the fat globules that may

remain in the butter.[3] A record of one experiment not included in the data given in this paper shows that the yield of membrane protein from the free buttermilk practically equalled that left in the butter. Although this is the result of only a single experiment, it indicates that the concentration of membrane material per unit of water may be as much as two and one-half times as great in the butter as in the free buttermilk. While it is interesting to speculate regarding the significance of this finding it must be admitted that it would be hazardous to suggest that it supports exclusively either the emulsion inversion theory or a modified foam theory that would allow for the release of at least one-half of the fat globule "hulls" during churning. Both theories would account for a greater concentration of "membrane" material in the butter; the inversion theory does not require a complete denuding of the fat surfaces before they can break through and allow true coalescence of the fat to occur and the agglutination of the fat globules that is supposed to occur in the foam theory is regarded as caused by a coagulation of the membrane substance, and not by its removal. The ultramicroscopic appearance of the colloidal sols of membrane material can not be interpreted as favoring a coagulation of membrane protein during churning even though some coagulation of colloidal particles was evident in some of the mechanically fractionated portions. The largest part of the colloidal material exhibited no evidence of coagulation. In fact this aspect of the foam theory of churning is rendered untenable by this result. Moreover the absolute necessity of foaming in order to produce butter, as claimed by Rahn, is completely overthrown by the experiments of Mohr and Brockmann (34) who showed that it is possible to churn both aged and acid creams in full churns with the complete exclusion of air, as well as creams obtained from buttermilks, all of which Rahn has regarded as incapable of accomplishment. Similar results have been obtained by us in, as yet, unpublished experiments.

## CONCLUSIONS

(1) The material most closely adsorbed on the surface of the fat globules in cow's milk is composed of a mixture of protein and phospholipides.

(2) The protein possesses the properties of both a hydrophilic and a hydrophobic colloid. Its physical properties, percentage of nitrogen, sulfur, and phosphorus do not correspond with those of any other milk protein. It constitutes the major part of the so-called fat globule membrane.

(3) The phospholipides constituting a part of the membrane are a mixture of mono-amino- and diamino-compounds.

[3] *Cf.*, King's (30) conception of butter structure which seeks to reconcile a complete emulsion inversion theory and the foam theory of churning on the basis that butter consists of a continuous fat phase in which both fat globules surrounded by their membrane and water drops are dispersed.

(4) The isoelectric point of the fat globule membrane material lies at pH 3.9–4.0. Its exact relation to various dairy manufacturing operations remains to be determined.

(5) Calcium is not intimately associated with the material closely adsorbed on the surface of the fat globules in cow's milk.

(6) A large part of the fat globule membrane material is removed during churning.

(7) The ultramicroscopic appearance of aqueous membrane sols makes untenable the theory that coagulation of the fat membrane material occurs during churning.

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# THE LEUCOCYTE CONTENT OF MILK AS CORRELATED WITH BACTERIAL COUNT AND HYDROGEN ION CONCENTRATION FOR THE DETECTION OF MASTITIS*

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## INTRODUCTION

From the public health standpoint it is very desirable to develop some method which will indicate the presence of udder infections. Microscopic examination of cow's milk always reveals the presence of leucocytes or white blood cells. Some investigators have regarded the presence of excessive numbers of these cells in milk as indicative of udder inflammation. All leucocytes in milk, however, cannot be regarded as pus cells because they are always found in milk. Leucocytes normally occur in the blood of animals and from there migrate into the body tissues; therefore, leucocytes may be called pus cells only when they come from suppurative discharges.

The dairy cow's udder is very susceptible to infection. Cows suffering from mastitis usually produce milk containing unusually large numbers of leucocytes. Therefore, large numbers of leucocytes in milk might serve as a reliable index to mastitis. When the leucocytes are accompanied by streptococci or other infective organisms, there can be little question concerning the presence of an active infection and an examination of the udder usually will reveal physical symptoms of inflammation. The present paper presents data concerning the leucocyte content of milk in relation to the health condition of the udder. Comparison is made between the leucocyte content of milk and other laboratory methods used for determining mastitis.

## HISTORICAL

Many studies have been published, both locally and abroad, which present data showing the number and possible significance of leucocytes in milk. Some investigators maintain that wide fluctuations in the leucocyte content of milk have little or no significance. Other investigators contend that high leucocyte counts indicate udder infection and that low leucocyte counts can be obtained only from normal, healthy cows. It is not the intention of this paper to give a complete bibliography on this subject, but merely to cite a few pertinent references.

The cellular content of milk has been shown to fluctuate between wide limits. Breed (3 and 4), using the Prescott and Breed (10) technique.

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examined the milk from 122 cows generally regarded as normal. He found the average cell count was 868,000 per cc., and that 27 of the cows gave milk with a count of over 1,000,000 per cc. He reported obtaining apparently normal milk strippings from a single teat which contained 54,000,000 leucocytes per cc.; no streptococci or other undesirable bacteria could be found in this milk. The milk was reported to appear normal and was sold for normal milk. He concluded that "leucocytes, even in fairly large numbers, *i.e.*, millions per cc., may be present in milk that is normal so far as is evident from ordinary chemical or bacteriological examination." Copeland and Olsen (7), using the same technique as was employed by Breed, found an average cell count of 657,000 per cc. for forty cows.

Hastings and Hoffman (8) studied two cows producing milk containing large numbers of bacteria and leucocytes. The authors believed it was possible that the animals had suffered from mastitis at some previous time and had continued to harbor the organisms after recovering. "In other words, cows are possibly able to become 'carriers of bacteria' after attacks of contagious garget just as human beings become 'carriers' of typhoid."

Experimentally, high leucocyte counts may be produced artificially; for example, Hoffman (9) found that boric acid, sodium chloride, and even distilled water, when injected into the udder, produced a marked increase in the number of leucocytes in the milk. The udder became inflamed and showed swelling with fever. He often noted a decrease in bacterial count with an increase in leucocytes and suggests that this may be due to phagocytosis. Further, Cooledge (6), and later Tweed (11), found a high cellular count in milk obtained from *Bacterium abortus* infected udders.

Campbell (5) found that the leucocyte count and sediment was low in milk taken from a young cow in her first lactation period. No streptococci were found on examination of the sediment. He points out that there seems to be some relationship between the presence of streptococci and the number of leucocytes in milk.

Baker and Breed (1) and Tweed (11) found a close relationship to exist between decreasing hydrogen-ion concentration of the fresh milk and an abnormal number of leucocytes. They attribute the decreasing hydrogen ion concentration to the entrance of the alkaline substances of the blood into the milk. Baker and Breed further concluded that "samples containing large numbers of leucocytes usually, though not invariably, contained streptococci as well."

Baker and Van Slyke (2) developed a colorimetric method for detecting abnormal milk. They published results of the examination of 570 samples of market milk and reported the detection of watered milk and also milk containing excessive numbers of leucocytes.

## PURPOSE OF THIS INVESTIGATION

The foregoing references indicate a lack of agreement relative to the sanitary significance of the numbers of leucocytes in milk. It is the purpose of this paper to present data which have been secured in detailed studies which have been made on the dairy herd of the University of Idaho. The herd is composed of Jerseys and Holsteins, and is free from Bang's abortion disease and tuberculosis as attested by successive tests.

## METHOD

The animals used in this study were constantly under veterinary supervision. The basis of selection was that young cows with a negative history of udder infection of any degree and which produced milk of low bacterial content were termed normal cows for the purpose of this study.

Other animals showing definite clinical symptoms of udder infection such as swelling, fever, hardening of the gland, or the presence of flakes in the milk were selected for study in the diseased group.

Samples of middle milk, representing each quarter of the cow's udder, were milked directly into sterile test tubes and brought directly to the laboratory where the reported tests were made as soon as possible.

The method of Prescott and Breed (10) for determining the cell content of milk was used throughout this paper. It consists of smearing 0.01 cc. of milk over an area of one square centimeter and staining. The cells were then counted by means of the oil immersion objective of a standardized microscope.

The hydrogen ion concentration has been determined by both the colorimetric method of Baker and Van Slyke (2) and by the quinhydrone electrode.

Bacterial counts were determined by plating the milk on standard nutrient agar and also nutrient agar to which had been added one per cent dextrose and not less than five per cent of sterile defibrinated horse blood.

## EXPERIMENTAL RESULTS

Table 1 presents data showing the relationship of the leucocyte counts to the hydrogen-ion concentration, and the numbers of bacteria per cc. of middle milk from healthy and diseased cows. An examination of these data shows that the bacterial counts on milk from normal udders is much lower than that on milk from diseased udders. The bacterial counts pertaining to healthy cows show that 86 per cent of the counts on plain agar and 78 per cent on blood agar were less than 1,000 per cc. The average count on plain agar was 1,000 per cc. and the average on blood agar was 1,600 per cc. The highest bacterial count found in milk from an apparently normal udder was 32,000 on cow No. 164. This count was obtained with

TABLE 1

*Analysis of Milk in Relation to Udder Infection*

| | NO. OF COW | TEAT | BACTERIA PER CC. | | LEUCOCYTES PER CC. | pH QUINHYDRONE | pH BROM CRESOL PURPLE |
|---|---|---|---|---|---|---|---|
| | | | Plain Agar | Blood Agar | | | |
| NORMAL COWS | 80 | R F | 500 | 100 | 20,000 | 6.8 | 6.5 – |
| | | R R | 0 | 300 | 44,000 | 6.8 | 6.5 – |
| | | L R | 0 | 200 | 132,000 | 6.8 | 6.5 – |
| | | L F | 300 | 300 | 44,000 | 6.9 | 6.5 –6.6 |
| | 83 | R F | 800 | 400 | 21,000 | 6.75 | 6.67–6.75 |
| | | R R | 600 | 400 | 25,000 | 6.8 | 6.67–6.75 |
| | | L R | 100 | 600 | 44,000 | 6.8 | 6.82–6.90 |
| | | L F | 0 | 300 | 21,000 | 6.8 | 6.82–6.90 |
| | 89 | R F | 5,200 | 3,000 | 22,000 | 6.7 | 6.5 |
| | | R R | 1,000 | 300 | 21,000 | 6.8 | 6.5 – |
| | | L R | 3,600 | 3,000 | 44,000 | 6.8 | 6.5 – |
| | | L F | 1,000 | 600 | 44,000 | 6.8 | 6.5 – |
| | 94 | R F | 200 | 300 | 88,000 | 6.7 | 6.67–6.75 |
| | | R R | 7,600 | 4,000 | 44,000 | 6.7 | 6.67–6.75 |
| | | L R | 400 | 200 | 44,000 | 6.7 | 6.67–6.75 |
| | | L F | 400 | 600 | 20,000 | 6.7 | 6.67–6.75 |
| | 150 | R F | 100 | 200 | 20,000 | 6.63 | 6.5 |
| | | R R | 200 | 100 | 20,000 | 6.66 | 6.5 – |
| | | L R | 0 | 100 | 40,000 | 6.66 | 6.5 – |
| | | L F | 350 | 1,200 | 20,000 | 6.66 | 6.5 – |
| | 156 | R F | 0 | 100 | 44,000 | 6.8 | 6.5 – |
| | | R R | 0 | 200 | 44,000 | 6.8 | 6.5 – |
| | | L R | 0 | 200 | 20,000 | 6.8 | 6.5 – |
| | | L F | 100 | 300 | 21,000 | ..... | 6.5 –6.6 |
| | 157 | R F | 2,400 | 2,400 | 88,000 | 6.85 | 6.5 – |
| | | R R | 100 | 300 | 122,000 | 6.85 | 6.5 – |
| | | L R | 100 | 400 | 88,000 | 6.75 | 6.5 – |
| | | L F | 0 | 200 | 88,000 | 6.8 | 6.5 – |
| | 159 | R F | 350 | 1,400 | 44,000 | 6.65 | 6.5 – |
| | | R R | 100 | 100 | 20,000 | 6.7 | 6.5 – |
| | | L R | 100 | 200 | 21,000 | ..... | 6.5 – |
| | | L F | 0 | 0 | 44,000 | 6.65 | 6.5 – |
| | 164 | R F | 200 | 100 | 40,000 | 6.7 | 6.5 – |
| | | R R | 8,800 | 32,000 | 44,000 | 6.7 | 6.5 – |
| | | L R | 100 | 0 | 44,000 | 6.8 | 6.5 – |
| | | L F | 800 | 4,800 | 20,000 | 6.8 | 6.5 – |
| DISEASED COWS | 61 | R F | 2,800 | 3,000 | 88,000 | 6.7 | 6.98–7.05 |
| | | R R | 3,800 | 120,000 | 13,200,000 | 6.85 | 7.13+ |
| | | L R | 600 | 6,000 | 2,640,000 | 6.9 | 6.67–6.75 |
| | | L F | 5,000 | 200,000 | 13,640,000 | 7.0 | 7.13+ |
| | 67 | R F | 6,800 | 65,000 | 660,000 | 6.75 | 6.67–6.75 |
| | | R R | 600 | 800 | 88,000 | 6.7 | 6.67–6.75 |
| | | L R | 15,000 | 50,000 | 704,000 | 6.75 | 6.67–6.75 |
| | | L F | 50,000 | 46,000 | 484,000 | 6.8 | 6.98–7.05 |
| | 84 | R F | 400 | 400 | 44,000 | 6.7 | 6.5 – |
| | | R R | 300 | 800 | 21,000 | 6.7 | 6.5 – |
| | | L R | 8,000 | 14,000 | 1,496,000 | 7.2 | 7.13+ |
| | | L F | 700 | 700 | 21,000 | 6.8 | 6.5 –6.6 |
| | 88 | R F | 48,000 | 50,000 | 380,000 | 6.75 | 6.5 –6.6 |
| | | R R | 60,000 | 60,000 | 11,880,000 | 7.0 | 7.13+ |
| | | L R | 34,000 | 14,000 | 132,000 | 6.8 | 6.5 – |
| | | L F | 3,600 | 1,000 | 1,320,000 | 6.8 | 6.6 –6.67 |

blood agar and was so much higher than the plain agar count that it might be regarded with suspicion. The leucocyte count and pH value, however, appeared normal.

Milk from cows suffering with mastitis had a high bacterial count when compared with milk from normal udders. Sixty-nine per cent of the samples from infected udders contained more than 2,000 bacteria per cc. on either plain or blood agar. The average count on plain agar was 15,000 per cc., and 40,000 per cc. on blood agar. The real significance of these counts, however, is illustrated more strikingly by pointing out the extremely high counts obtained from the infected quarters, which ranged from 14,000 to 200,000 per cc.

Bacterial counts on plain and blood agar compare favorably, but the colonies on blood agar were much easier to count. This was because most colonies appearing on plain agar from milk drawn from infected quarters were so small that they were hardly visible with a standard 5X lens. Often the medium in these plates merely looked cloudy to the naked eye. Well developed colonies which were easily counted with the naked eye, instead of the minute "pin point" colonies, appeared on blood agar. Excessive bacterial counts on blood agar, from aseptically drawn milk samples, were always correlated with udder infection.

The leucocyte content of milk from both normal and diseased quarters shows wide variations. Milk from infected quarters has a much higher leucocyte content than milk from normal quarters. This higher leucocyte count is not confined to the diseased quarters since a less striking increase is also noted in the quarters which are apparently normal. The average leucocyte count on milk from six normal cows (shown in table 2) was found to be 43,000 per cc., whereas the count on milk from seven diseased cows was found to be 3,000,000 per cc. The enormous numbers of leucocytes in milk from infected quarters serve as a more striking indication of infection, because a fluctuation of from one-half million to several millions of leucocytes per cc. may be induced by infection, whereas the bacterial count may be increased by only several thousand. The fact that the leucocyte content of milk remains constant after it is drawn adds to the reliability. These data in table 1 show a general relationship to exist between the leucocyte and the bacterial counts.

The hydrogen ion concentration of middle milk from normal and diseased udders is also given in table 1. The pH values were determined by the Brom Cresol Purple colorimetric method (2) and by the quinhydrone electrode. Middle milk from normal cows usually had a pH value of 6.6 to 6.8, according to the quinhydrone electrode, and 6.5 to 6.8 by the Brom Cresol Purple method. Milk from cows suffering from severe udder infections was usually neutral or slightly alkaline in reaction. Data obtained by both methods of determining pH correlate with the leucocyte and bac-

TABLE 2

*Frequent Determinations of the Leucocyte Content of the Milk of Individual Animals*

| | COW NO. | TEAT | LEUCOCYTES PER CC. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1st | 2nd | 3rd | 4th | 5th | 6th |
| NORMAL COWS | 83 | R F | 88,000 | 132,000 | 88,000 | 88,000 | 22,000 | 44,000 |
| | | R R | 22,000 | 44,000 | 44,000 | 44,000 | 44,000 | 44,000 |
| | | L R | 44,000 | 88,000 | 66,000 | 44,000 | 44,000 | 22,000 |
| | | L F | 22,000 | 88,000 | 132,000 | 88,000 | 132,000 | 88,000 |
| | 2X | R F | 11,000 | 22,000 | 44,000 | 22,000 | 22,000 | 44,000 |
| | | R R | 8,800 | 22,000 | 22,000 | 8,800 | 22,000 | 22,000 |
| | | L R | 44,000 | 22,000 | 22,000 | 22,000 | 44,000 | 44,000 |
| | | L F | 11,000 | 22,000 | 22,000 | 44,000 | 22,000 | 22,000 |
| | 8X | R F | 22,000 | 11,000 | 22,000 | 11,000 | 22,000 | 11,000 |
| | | R R | 22,000 | 22,000 | 8,800 | 22,000 | 11,000 | 22,000 |
| | | L R | 22,000 | 11,000 | 8,800 | 22,000 | 8,800 | 11,000 |
| | | L F | 22,000 | 22,000 | 8,800 | 11,000 | 11,000 | 22,000 |
| | 158 | R F | 11,000 | 22,000 | 44,000 | 44,000 | 22,000 | 22,000 |
| | | R R | 22,000 | 8,800 | 8,800 | 22,000 | 8,800 | 8,800 |
| | | L R | 44,000 | 44,000 | 44,000 | 22,000 | 22,000 | 44,000 |
| | | L F | 44,000 | 44,000 | 44,000 | 44,000 | 44,000 | 44,000 |
| | 159 | R F | 44,000 | 132,000 | 44,000 | 22,000 | 44,000 | 22,000 |
| | | R R | 22,000 | 44,000 | 44,000 | 44,000 | 44,000 | 44,000 |
| | | L R | 44,000 | 44,000 | 44,000 | 44,000 | 44,000 | 44,000 |
| | | L F | 88,000 | 44,000 | 88,000 | 44,000 | 88,000 | 44,000 |
| | 160 | R F | 44,000 | 44,000 | 44,000 | 22,000 | 44,000 | 22,000 |
| | | R R | 44,000 | 22,000 | 22,000 | 44,000 | 22,000 | 22,000 |
| | | L R | 44,000 | 22,000 | 44,000 | 22,000 | 44,000 | 44,000 |
| | | L F | 44,000 | 44,000 | 11,000 | 44,000 | 88,000 | 44,000 |
| DISEASED COWS | 54 | R F | 4,840,000 | 2,508,000 | 3,100,000 | 3,784,000 | 3,652,000 | 3,608,000 |
| | | R R | 1,144,000 | 88,000 | 352,000 | 308,000 | 1,144,000 | 486,000 |
| | | L R | 2,948,000 | 5,192,000 | 2,876,000 | 2,596,000 | 5,016,000 | 264,000 |
| | | L F | 10,120,000 | 37,400,000 | 5,016,000 | 9,812,000 | 1,804,000 | 3,432,000 |
| | 55 | R F | 1,320,000 | 484,000 | 1,056,000 | 784,000 | 1,672,000 | 924,000 |
| | | R R | 246,000 | 220,000 | 484,000 | 484,000 | 352,000 | 308,000 |
| | | L R | 220,000 | 748,000 | 748,000 | 264,000 | 15,840,000 | 24,000,000 |
| | | L F | 220,000 | 396,000 | 232,000 | 264,000 | 176,000 | 704,000 |
| | 65 | R F | 792,000 | 1,232,000 | 528,000 | 704,000 | 4,488,000 | 2,156,000 |
| | | R R | 44,000 | 132,000 | 88,000 | 44,000 | 88,000 | 1,716,000 |
| | | L R | 1,364,000 | 22,000,000 | 28,040,000 | 17,600,000 | 1,056,000 | 3,124,000 |
| | | L F | 704,000 | 17,160,000 | 13,640,000 | 14,520,000 | 2,288,000 | 5,128,000 |
| | 71 | R F | 132,000 | 740,000 | 616,000 | 1,188,000 | 1,452,000 | 1,454,000 |
| | | R R | 440,000 | 396,000 | 640,000 | 640,000 | 1,260,000 | 1,002,000 |
| | | L R | 484,000 | 528,000 | 396,000 | 680,000 | 1,364,000 | 1,188,000 |
| | | L F | 264,000 | 572,000 | 484,000 | 880,000 | 1,100,000 | 1,760,000 |
| | 80 | R F | 264,000 | 1,760,000 | 484,000 | 132,000 | 176,000 | 264,000 |
| | | R R | 440,000 | 220,000 | 352,000 | 308,000 | 264,000 | 132,000 |
| | | L R | 1,472,000 | 2,464,000 | 1,056,000 | 14,080,000 | 4,400,000 | 20,680,000 |
| | | L F | 264,000 | 968,000 | 440,000 | 352,000 | 44,000 | 176,000 |
| | 88 | R F | 264,000 | 748,000 | 132,000 | 880,000 | 88,000 | 22,000,000 |
| | | R R | 220,000 | 1,012,000 | 768,000 | 308,000 | 484,000 | 352,000 |
| | | L R | 968,000 | 484,000 | 1,100,000 | 264,000 | 176,000 | 352,000 |
| | | L F | 484,000 | 264,000 | 176,000 | 572,000 | 220,000 | 44,000 |
| | 141 | R F | 18,040,000 | 3,564,000 | 8,800,000 | 352,000 | 4,000,000 | 2,200,000 |
| | | R R | 1,232,000 | 528,000 | 924,000 | 660,000 | 924,000 | 352,000 |
| | | L R | 836,000 | 968,000 | 572,000 | 660,000 | 2,112,000 | 88,000 |
| | | L F | 1,276,000 | 396,000 | 572,000 | 528,000 | 660,000 | 396,000 |

terial content of milk as an index to mastitis. The changes in reaction, except in severe cases, were so slight, however, that they were difficult to determine by the colorimetric method. Further, the pH values might be misleading if the milk had been watered or if it contained alkaline substances. Considering the data presented in this table, it seems apparent that of all the laboratory methods reported here, the leucocyte content of milk is the truest indicator of mastitis.

Table 2 presents the results of repeated leucocyte counts made on milk from normal and diseased udders. These counts were made regularly over a period of several months. Nearly 800 determinations were made, but space will not permit presentation of all the accumulated data. The data here presented represents repeated leucocyte counts on middle milk samples, from six normal cows that had never been known to suffer from mastitis, and from seven diseased cows. Analysis of these data shows that of the 144 counts recorded from representative normal cows, 94 per cent of the samples contained more than 10,000 leucocytes per cc.; 11 per cent more than 50,000; and only 3 per cent more than 100,000. Of the 168 leucocyte counts pertaining to seven diseased cows, 99 per cent showed more than 50,000 leucocytes per cc.; 96 per cent contained more than 100,000; and 40 per cent contained more than 1,000,000.

TABLE 3

*Summary of 758 Leucocyte Counts on Milk from Normal Cows and Cows Suffering with Mastitis*

| | LESS THAN 10,000 PER CC. | FROM 10,000 TO 50,000 | FROM 50,000 TO 100,000 | FROM 100,000 TO 1,000,000 | OVER 1,000,000 |
|---|---|---|---|---|---|
| Normal Cows | 6.3% | 83.4% | 7.6% | 2.7% | 0.0% |
| Diseased Cows | 0.0% | 0.3% | 0.7% | 34.5% | 64.5% |

Table 3 is a summarized report of the distribution of 758 leucocyte counts from the milk of normal and diseased cows. An examination of these data reveals that approximately 90 per cent of the leucocyte counts from normal udders contains less than 50,000 leucocytes per cc. Only 2.7 per cent of the counts exceed 100,000. In contrast with these data are the counts obtained on milk from diseased cows. Here it will be found that only one per cent of the samples contains less than 100,000 leucocytes per cc. Since these counts represent all of the individual quarters of each cow, regardless of the apparent health condition, it seems logical to regard such a large number of determinations as a reliable basis for interpreting the significance of the leucocyte count of milk from normal and diseased cows.

Since approximately 90 per cent of all counts on milk from individual quarters of normal cows contained less than 50,000 leucocytes per cc., it is

apparent that a composite sample representing the four quarters of a cow would generally contain less than 50,000. Again, 99 per cent of the samples from individual quarters of diseased cows contained more than 100,000 per cc.; therefore, a composite milk sample from diseased cows would nearly always exceed this amount. The periodic determination of the leucocyte content of milk samples from each cow in a herd should be a reliable guide in locating cases of mastitis.

## SUMMARY

This paper presents experimental data concerning the laboratory diagnosis of mastitis in cows. Aseptically drawn milk samples representing the individual quarters of each udder were used. The following conclusions appear valid:

(1) Of all the laboratory methods used in this investigation, the leucocyte content of milk is the truest indicator of mastitis.

(2) Bacterial counts on blood agar were more reliable for detecting udder infections than those on plain agar because plain agar often failed to produce distinct colonies from the organisms which were responsible for the infection.

(3) Hydrogen ion determinations by the quinhydrone and Brom Cresol Purple methods correlate with other laboratory methods used to determine udder infections. The changes in reaction, however, are so slight, except in extreme cases, that the results are difficult to interpret.

(4) The leucocyte count seems to be the most reliable index of udder infection, due to the enormous numbers of leucocytes encountered in milk from infected quarters, and the generally high leucocyte content of milk drawn from apparently normal quarters of the same udders. The fact that the leucocyte content of milk remains constant after it is drawn adds to its reliability.

(5) Analysis of 758 leucocyte counts seems to warrant the conclusion that milk from normal udders usually contains less than 50,000 leucocytes per cc., whereas milk from infected udders almost invariably contains more than 100,000 leucocytes per cc.

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# DRYING UP COWS AND THE EFFECT OF DIFFERENT METHODS UPON MILK PRODUCTION[1]

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The dairyman is often confronted with the problem of drying up persistent producing cows. The practical importance of this problem is illustrated by the fact that, with the exception of feeding, more questions are asked of the Dairy Husbandry Division at the University of Minnesota regarding drying up cows than any other single subject.

A questionnaire prepared by the authors and answered by 236 dairy farmers revealed a wide range in opinion and practises regarding the subject under consideration. Of those reporting 76 per cent follow the practice of intermittent milking when drying up their cows, 16 per cent follow the plan of sudden and complete cessation of milking, and 8 per cent use incomplete milking. One-third reported difficulty in drying up their highest producing cows.

Considering the importance of the problem it is surprising that no controlled experimental work has been reported in which the object was to determine the most satisfactory procedure to follow in drying up cows.

One of the authors (1) states that, "If a cow is producing as little as ten pounds per day, milking can be stopped at any time and no harm will result. The udder should not be milked out at all. It will fill up for a few days and then the milk contained is gradually resorbed and no harm will result in any case. If a cow is producing more than this amount of milk, it is advisable to first cut down on her feed." This was based upon personal observations extending over several years during which time the practise of drying up cows by complete cessation of milking was followed with no harmful effects.

That milk secretion is stimulated by hormones has been shown by a number of workers. This stimulus is most pronounced for three or four months following parturition and with most cows is markedly diminished in the later part of the lactation period. With persistent producers this stimulus together with the nervous stimulation resulting from the act of milking is sufficient to maintain a fair level of milk production until the next parturition unless some special effort is made to stop secretion. Drying up such cows has been considered a difficult problem by many dairymen.

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[1] The data in this paper are taken from a thesis presented to the Graduate Faculty, University of Minnesota, by Ralph Wayne in partial fulfillment of the requirements for the degree of Master of Science. Published with the approval of the Director as Paper 1096 Journal Series, Minnesota Experiment Station.

The primary factors to be considered in a comparison of methods of drying up cows are, the effects upon the cow, the time required to dry up the cow, and the quantity and quality of milk yielded in subsequent lactations. Some concrete basis upon which to compare effects of different methods of drying up cows is necessary in order to arrive at significant conclusions.

Two procedures lend themselves for use in making such comparisons. One of these consists in drying off all four quarters of the udder by the same method. The production of the fore part of the next lactation is then compared with corresponding periods of previous lactations when a different method was used in drying off the cow. This method can be criticized on the ground that two different lactations are compared and such factors as level of feeding, age, and condition of the cow, which affect milk yield are not controlled. Were this method followed a large number of cows would have to be used in order to be certain that these factors, rather than the methods used in drying off the cows, were not responsible for the results obtained.

Bitting (2) and Rigor (3) have shown that the quarters of the bovine mammary gland are anatomically separate. Upon this fact was based a second technique which was used in securing some of the most significant data gathered during this investigation. This second technique of drying off one-half of the udder by one method and the other by another eliminates the influence of age, feed level and condition, and the disturbing factors encountered under the first possible plan of procedure mentioned. As the two halves of the mammary system are separate anatomically as well as physiologically all factors considered in this investigation can be compared when this technique is used.

When following this plan the cows were maintained on the same level of feeding during the drying off period as during the period just preceding. Therefore, no effect upon the rate of drying off could be attributed to a decrease in feed consumption. Each quarter of the udder was considered as a unit. All milking was done by hand.

Experiment 1. *The effect of delayed milking of a cow in the early stage of lactation*

The cow used was in the second month of lactation and no milk was drawn for 11.5 days. Data were also taken from the same cow to determine if milking one half of the udder stimulates milk secretion in the other gland, when in the dormant state.

A two year old Holstein was used that had been in milk 58 days and was milking an average of 18.2 pounds daily when milking was discontinued. The udder became quite distended and the cow appeared uneasy for two or three days after which the pressure within the gland gradually decreased

resulting in the udder becoming smaller. After a delay in milking of 11.5 days all quarters were milked dry. At that time 5.4 pounds of secretion were drawn, which was of a dark amber color with a salty taste. The lactose content was only 0.43 per cent and the pH showed the extremely high point of 7.34. The left half was then milked at 12 hour intervals while milking of the right half was delayed 9.5 days more after which it was also milked at 12 hour intervals. The yield and composition of milk from the left half gradually returned to normal when milked at regular 12 hour intervals, although the yield decreased to as low as 1.0 pound for two or three milkings. Following the second delayed milking of the right half, 2.5 pounds of secretion were drawn. It had the same characteristics as that drawn at the close of the previous delayed milking. Milking of the left half during this delayed milking of the right half had no stimulating effect on the latter. When milked at 12 hour intervals production of the right half decreased to as low as 0.8 pound for two or three milkings and then gradually increased. However, the level of production never reached over 45 per cent of the preliminary level, even after twenty days of regular twice daily milking. The composition of the milk returned to normal.

Results of this experiment show that no harmful effects resulted when milking of a cow in the second month of her lactation was delayed 11.5 days. A comparatively small amount of milk was found in the udder following this delay. It was demonstrated that the normal level of production can again be attained by milking at regular 12 hour intervals if the delay has not exceeded 11.5 days. It was also shown in this experiment that milking of one-half does not influence secretion of milk in the other half of the udder. This fact was given consideration in the technique as reported for experiment 4. One half may remain dormant even though the other is milked and in an active state of secretion.

Experiment 2. *Incomplete milking as a method for drying off cows.*

The purpose of this experiment was to study incomplete milking as a method for drying up cows. The effect on the udder and the rate of decline of milk yield were considered. Two cows were used; a purebred Jersey, Cow 173, 173 days advanced in her second lactation, and producing about 8 pounds daily, and Cow 146, a grade Holstein in her tenth month of lactation and producing about 18 pounds daily.

Cow 173 was dried off by leaving about one-half of the milk present in the udder at milking time. All quarters were milked dry during a preliminary period after which approximately one-half of the milk present in the udder was removed at twelve hour intervals for eight milkings. The interval between incomplete milkings was then progressively increased until the delayed milking was extended to seven days at which time the udder was milked dry. For the first eight milkings the udder appeared full and some-

what distended. Following this the udder gradually became smaller with less and less milk being present at each milking.

Two methods of drying off were compared on cow 146. All quarters were milked dry during a preliminary period of four milkings. Milking of the left half was then discontinued. In case of the right half approximately one-half of the milk present in the gland at milking time was removed at 24 hour intervals for ten days. The interval between incomplete milking was then progressively increased until it reached 96 hours when all quarters were milked dry.

The left half became quite firm and distended upon cessation of milking for three days after which the pressure within the gland decreased rapidly accompanied by a decrease in the size of the gland. Considerable pressure was present in the right half at milking time for the first four days of incomplete milking. This half then gradually decreased in size, although at a much slower rate than the left half, being dried off by complete cessation of milking. Nineteen days following the preliminary period all quarters were milked dry. At that time about twice as much milk was present in the right half as in the left half.

This experiment shows that it requires a longer period of time to dry a cow off by incomplete milking than by complete cessation of milking.

Experiment 3. *Complete cessation of milking as a method for drying off cows*

The purpose of this experiment was to note the characteristic changes in the milk, the gross changes in the udder, and the effects upon the production during the next lactation when milking of cows is suddenly terminated.

Details concerning the cows used in this experiment at the time they were dried off are given in table 1:

TABLE 1

*Data on cows dried off by complete cessation of milking*

| NUMBER OF COW | AVERAGE DAILY PRODUCTION POUNDS | DAYS IN MILK DURING PRESENT LACTATION | LACTATION PERIOD | AGE YRS. | AGE MO. | BREED |
|---|---|---|---|---|---|---|
| 153 | 16.4 | 290 | 6 | 8 | 3 | Purebred Jersey |
| 167 | 6.8 | 471 | 2 | 4 | 10 | " " |
| 382 | 6.9 | 392 | 3 | 5 | 10 | " Holstein |
| 542 | 10.2 | 222 | 3 | 5 | 9 | " Guernsey |
| 392 | 22.7 | 172 | 2 | 4 | 10 | " Holstein |
| 548 | 5.9 | 224 | 2 | 4 | 10 | " Guernsey |

Milking was discontinued following a preliminary milking of four milkings. Cows 153, 167 and 542 were not milked again until after parturition. The remaining cows 382, 392, and 548 were milked dry following a delay in

milking of seven, sixteen, and ten days respectively. Lactose and pH determinations were made of the milk drawn.

When milking was discontinued the udders became distended and firm. In only one case was this severe enough to cause discomfort to the cow. In two or three days pressure within the gland began to decrease. The milk drawn following an extended delay in milking was similar in all respects to that described in Experiment 1. Lactose percentages as low as 0.14 per cent and pH as high as 7.59 were observed. Following a delay in milking of 16 days only 1.6 pounds of secretion were secured from cow 392 although she produced an average of 22.7 pounds during the preliminary period before drying off was started.

Production records following the next parturition were obtained in the case of three cows. Comparisons with corresponding periods of previous lactations are presented in table 2. Only the production during the first part of the lactation was used in this comparison in order to avoid the influence of a difference in gestation. It is recognized that other factors as feed, and condition which influence milk production have not been controlled from one lactation to the next. However, if any marked effects were produced in drying off cows by complete cessation of milking it would be reflected on the production during the fore part of the next lactation. Ex-

TABLE 2

*Production during the first part of the lactation period following the drying off of the cow by complete cessation of milking compared with the corresponding period in previous lactations*

| METHOD USED IN DRYING OFF COW IN PREVIOUS LACTATION | AGE AT TIME OF PARTURITION | | MILK-YIELD | AVERAGE FAT PERCENTAGE | TOTAL FAT YIELD | NUMBER OF TIMES MILKED DAILY |
|---|---|---|---|---|---|---|
| | Years | Months | Pounds | | Pounds | |
| **Cow 167—first 209 days of lactations compared** | | | | | | |
| First lactation | 2 | 5 | 5363.3 | 6.47 | 347.1 | 3 |
| Intermittent milking | 3 | 6 | 5311.8 | 5.79 | 307.5 | 2 |
| Complete cessation of milking | 4 | 11 | 5532.2 | 5.67 | 313.7 | 2 |
| **Cow 382—first 86 days of lactations compared** | | | | | | |
| First lactation | 2 | 2 | 3655.5 | 3.16 | 115.5 | 3 |
| Intermittent milking | 3 | 8 | 3110.6 | 4.02 | 125.0 | 2 |
| Intermittent milking | 4 | 10 | 3970.7 | 3.25 | 129.0 | 2 |
| Complete cessation of milking | 6 | 4 | 4886.5 | 3.47 | 169.6 | 2 |
| **Cow 542—first 157 days of lactations compared** | | | | | | |
| First lactation | 2 | 8 | 4041.6 | 4.43 | 179.0 | 3 |
| Intermittent milking | 3 | 11 | 3887.5 | 5.03 | 195.5 | 2 |
| Intermittent milking | 5 | 2 | 4130.7 | 4.37 | 180.5 | 2 |
| Complete cessation of milking | 6 | 1 | 3892.3 | 5.23 | 203.5 | 2 |

amination of the figures show the ranges in production are within limits of variation caused by factors other than methods used in drying off the cows.

Experiment 4. *Complete cessation of milking compared with intermittent milking as methods for drying off cows*

The purpose of this experiment was to compare intermittent milking with complete cessation of milking as methods for drying off cows. As intermittent milking is a method extensively used it serves as a check for complete cessation of milking, a method less commonly used.

Information concerning the nine cows used in this experiment is given in table 3:

TABLE 3

*Data on cows with half of udder dried off by complete cessation of milking and other half by intermittent milking*

| COW NUMBER | AVE. DAILY PRODUCTION | DAYS IN MILK DURING PRESENT LACTATION | LACTATION PERIOD | AGE YRS. | AGE MO. | BREED |
|---|---|---|---|---|---|---|
| | *pounds* | | | | | |
| 70 | 14.7 | 366 | 3 | 5 | 2 | Grade Holstein |
| 73 | 14.7 | 352 | 3 | 5 | 1 | " " |
| 36 | 7.0 | 367 | 1 | 2 | 11 | " " |
| 138 | 12.3 | 238 | 2 | 4 | 11 | " Guernsey |
| 155 | 10.6 | 306 | 4 | 6 | 7 | Purebred Jersey |
| 536 | 10.1 | 338 | | 12 | 11 | " Guernsey |
| 538 | 16.4 | 221 | 4 | 6 | 10 | " " |
| 549 | 10.8 | 242 | 2 | 4 | 6 | " " |
| 551 | 8.5 | 192 | 2 | 4 | 4 | " " |

In all cases the right half was dried off by intermittent milking and the left half by complete cessation of milking. Weights of the milk produced by individual quarters were secured during four to twelve preliminary milkings to determine the production ratio of the two halves of the udder. Following the preliminary period the left half was not milked again until after parturition. The right half was milked at 24 hour intervals for three days and then at 48, 72, 96 and 168 hour intervals after which milking was discontinued until after parturition.

The milk yielded by each quarter during the 8th to 22nd milkings inclusive following parturition were used in calculating the production ratios of the two halves of the udder during that period. These ratios are compared in table 4 with those obtained previous to drying off.

This plan for comparing the effect on production in the next lactation of the two methods of drying off cows eliminates influences of other factors affecting production. Using eleven cows Turner (4) found that of the total production, the percentage produced by each gland remained nearly con-

stant from one lactation to the next. Accepting Turner's results, in this experiment it seemed sound to attribute to the methods used in drying off the cows, any marked change in the production ratio of the two halves during the next lactation. However it is recognized that Turner compared the production in one lactation with that in the next while in this experiment the production during the latter part of one lactation was compared with that of the fore part of another.

The data given in table 4 show that there was practically no change in the production ratios of the two halves from one lactation to the next. The slightly higher figure for the left half is not considered sufficient to be significant.

Certain variations were observed in the condition of the udder with different cows during the drying off period. In case of cows 70, 73, 138, 155, 536, 538, and 551 the left half became quite distended, and in some cases

FIG. 1. REAR VIEW OF THE UDDER OF COW 138, 16 DAYS AFTER DRYING UP WAS STARTED. Left half being dried up by complete cessation of milking and the right half by intermittent milking at progressive intervals of 24, 24, 24, 48, 72, 96, and 186 hours. This picture was taken just before the 186th hour interval milking of the right half. The left half was dry while 795 grams of secretion were obtained from the right half. The condition shown was characteristic of seven of the nine cows used in experiment 4.

quite firm when milking was discontinued. Resorption of milk from this half was sufficient three days following cessation of milking to relieve the pressure within the gland. By the tenth day the left half was much smaller than the right half. At that time the external appearance of the udders of all seven cows was similar to that shown in Fig. 1, which shows the left half dried off much quicker than the right half. The right half usually continued to secrete appreciable quantities of milk even after the intervals between milkings were considerably extended.

In case of cow 549 from external appearances there was no difference in the rate of drying off the right and left half. Cow 86 responded differently to the two methods than any of the other cows. The left half never became completely collapsed. This half remained larger and firmer than the right half and continued to contain considerable milk up until the time of parturition. During hypertrophy of the udder the left half enlarged much sooner

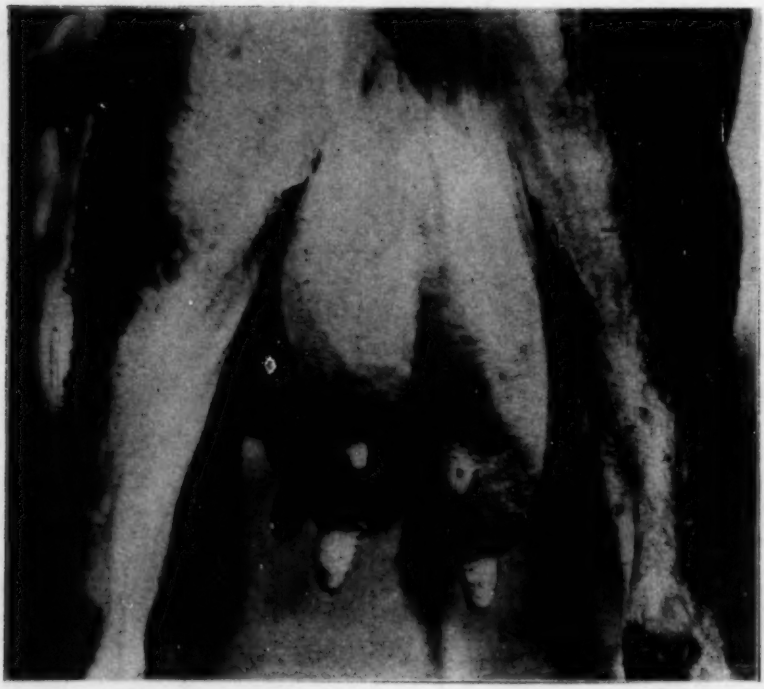

FIG. 2. REAR VIEW OF THE UDDER OF COW 138 FOUR DAYS AFTER PARTURITION.

The udder was milked dry five hours before the picture was taken. The picture shows the udder (also shown in figure 1) to be bilaterally symmetrical following parturition. The condition shown was observed in all nine of the cows used in experiment 4.

than the right half. However the glands were bilaterally symmetrical at the time of parturition.

In all cases the two halves of the udder were bilaterally symmetrical following parturition. The symmetry of the udder of cow 138 following parturition as shown in Fig. 2 was typical of all the cows.

## DISCUSSION

Results from this investigation show that complete cessation of milking can safely be recommended as a method for drying up cows. Cows were dried off in less time when this method was used than when incomplete and intermittent milking were employed. When milking is discontinued the udder will fill up until a pressure great enough to stop secretion is established in the gland. Rigor (3) has shown this pressure to be 25 millimeters of mercury. After secretion is stopped the milk is gradually resorbed from the gland until it becomes dry. The composition of the milk present in the gland during this resorption period is highly abnormal as Rigor has demonstrated in the research cited. The protein and pH of the secretion are greatly increased while the lactose percentage is markedly lowered. From a physiological standpoint it is a mistake to milk out a cow during the resorption period as this releases the pressure within the gland and secretion is again initiated resulting in a prolonged period of drying off.

TABLE 4

*Drying up cows by intermittent milking and by complete cessation of milking and the effects upon the milk yield in the next lactation period*

| COW NO. | AVERAGE DAILY PRODUCTION WHEN DRIED OFF | RIGHT HALF DRIED UP BY INTERMITTENT MILKING | | LEFT HALF DRIED UP BY COMPLETE CESSATION OF MILKING | |
|---|---|---|---|---|---|
| | | Proportion of total yielded by right half before drying up[1] | Proportion of total yielded by right half following parturition[2] | Proportion of total yielded by left half before drying up[1] | Proportion of total yielded by left half following parturition[2] |
| | *pounds* | *per cent* | *per cent* | *per cent* | *per cent* |
| 70 | 14.7 | 50.53 | 50.46 | 49.47 | 49.54 |
| 73 | 14.7 | 52.99 | 47.87 | 47.01 | 52.13 |
| 86 | 7.0 | 57.21 | 56.97 | 42.79 | 43.03 |
| 138 | 12.3 | 60.33 | 48.29 | 39.67 | 51.71 |
| 155 | 10.6 | 52.52 | 59.14 | 47.50 | 40.86 |
| 536 | 10.1 | 59.23 | 47.95 | 40.77 | 52.05 |
| 538 | 16.4 | 49.97 | 49.99 | 50.03 | 50.01 |
| 549 | 10.8 | 46.50 | 49.10 | 53.50 | 50.90 |
| 551 | 8.5 | 50.48 | 49.53 | 49.52 | 50.47 |
| Average | | 53.30 | 51.03 | 46.70 | 48.97 |

[1] Based on average of four to twelve preliminary milkings.

[2] Based on average of fourteen milkings, ninth to 22nd milkings, inclusive, after parturition.

SUMMARY

Results of an investigation using 18 cows in which three methods of drying off cows were studied and compared are reported. The methods considered were incomplete milking, intermittent milking, and complete cessation of milking. The data show that complete cessation of milking can safely be recommended as a method for drying off cows producing at least up to 20 pounds daily. Cows can be dried off in less time by this method than by either of the other two methods considered. No significant difference was noted in the quantity or quality of milk in the lactations following the drying up of cows by the three methods used.

Attention is called to the probable desirability of not disturbing the resorption phenomenon through intermittent or partial milking when drying off cows. Drying off cows through complete cessation of milking conserves leucocytes that may have an important function in resorption.

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# THE EFFECT OF VARIOUS METHODS FOR DRYING UP COWS ON THE BACTERIAL AND CELL CONTENT OF MILK[1]

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In connection with studies reported in the previous paper by Wayne, Eckles and Petersen (10), bacterial and cell counts of the milk were made at various intervals during the progress of the experiments. The producer of milk for the fluid milk market is interested in the effect which various procedures in herd management may have on the bacterial content of the milk where local ordinances require close adherence to bacterial standards.

Von Freudenreich (3) found less than the usual number of bacteria present in the udders of three cows which had not been milked for several weeks. On the other hand, Stocking (7) observed that the average bacterial count was higher when the strippings were not removed during the preceding milking. Steck (6) concluded that the failure to milk out the udder caused evident inflammation only in those quarters where the types of bacteria present favored such a change. Gorini (4) (5) was of the opinion that practices leading to the retention of milk had a significant effect on the bacterial flora.

Wallick and Levaditi (9) working with human milk found that there was a marked increase in leucocytes when there was a definite stopping of lactation. Varrier-Jones (8) maintained that cells were present in large numbers when the gland ceased to perform its proper function (as when the cow dried up). According to Bourgeois (1) the leucocyte formula of retained milk agreed with that of colostrum.

Many investigators agree with Ernst and his co-workers (2) that incomplete milking is conducive to udder troubles. This is a matter that merits especial attention and offers a fertile field for investigation.

The results presented in this paper are suggestive of changes which may be expected in bacterial and cell counts of milk under various conditions of milking, and following interruptions of different sorts. Following these preliminary trials, observations should be made over longer periods of time and on many animals.

## EXPERIMENTAL METHODS

The following methods were used in connection with these investigations:

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[1] The data in this paper are taken from a thesis presented to the Graduate Faculty, University of Minnesota, by Ralph Wayne in partial fulfillment of the requirements for the degree of Master of Science. Published with the approval of the Director as Paper 1097 Journal Series, Minnesota Experiment Station.

*Collection of samples:*

The milk from each quarter was drawn into separate sterile flasks. The udder and teats were washed with a clean cloth soaked in a freshly prepared solution of chloramine-T. A linen cloth, soaked in this solution, and then wrung out, was slipped over the mouth of the flask before the cotton plug was removed. The teat was extended through a small hole in the cloth. Milking was then performed as illustrated in plate 1. An additional sterile cotton plug was provided for each flask after the milking was completed. The samples were taken to the laboratory for immediate examination.

*Methods of Analysis:*

Bacterial: The standard agar plate method was used, with duplicate plates of the necessary dilutions. The plates were poured with beef infusion agar and incubated at 37° C. for 48 hours.

Cellular: The number of cells was determined by a slight modification of the standard microscopic (Breed) method. A platinum loop delivering 0.0062 gm. of milk was used in measuring the amount of milk spread over a square centimeter of surface on an ordinary glass slide. Duplicate smears were made. Newman's stain, formula II, was employed in most of the examinations, otherwise the usual xylene, alcohol, aqueous methylene blue series. Ten fields of each smear were counted under the oil immersion lens.

## PRESENTATION OF DATA

The data obtained in these studies are summarized in a number of tables which follow. A representative data sheet for one cow is given as table 1 to indicate the material available for each cow for computation in the tables presented subsequently.

In table 1, it will be noted that the usual differences in bacterial and cell counts in various quarters of the udder are evident. Another fact is clear, namely, that there are wide fluctuations in count from milking to milking of the same quarter even in the preliminary, normal, milking periods. This is significant in considering comparisons between counts obtained during experimental periods as brought out later.

## EFFECT OF INCOMPLETE AND INTERMITTENT MILKING

Cow 173.—She had been in milk for 173 days. Counts were made on three complete preliminary milkings at 12 hour intervals. At each of the eight milkings following, 12 hours apart, approximately one-half of the milk was drawn from each quarter. Then 24 hours elapsed before the next milking when one-half of the milk was drawn. This was followed by similar incomplete milkings at intervals of 24 hours, 2 days (twice), 3 days, and finally 7 days after which all the milk was drawn.

TABLE 1

*Effects of intermittent milking and complete cessation of milking on bacterial and cell counts of milk*

Cow 155

| DATE | BACTERIAL COUNT | | | | CELL COUNT | | | |
|---|---|---|---|---|---|---|---|---|
| | RF | RH | LF | LH | RF | RH | LF | LH |
| | *per cent.* | *per cent.* | *per cent.* | *per cent.* | *per cc.* | *per cc.* | *per cc.* | *per cc.* |
| **Preliminary Period** | | | | | | | | |
| 1930 7–11 PM | 20 | 5 | 1080 | 35 | 1,431,000 | 560,000 | 1,990,000 | 373,000 |
| 12 AM | 320 | 100 | 3400 | 95 | 809,000 | 311,000 | 3,359,000 | 311,000 |
| PM | 10 | 0 | 1600 | 5 | 498,000 | 622,000 | 4,665,000 | 311,000 |
| 13 AM | 160 | 0 | 1260 | 10 | 3,048,000 | 1,182,000 | 4,603,000 | 498,000 |
| PM | 175 | 20 | 450 | 30 | 1,057,000 | 995,000 | 3,919,000 | 498,000 |
| 14 AM | 500 | 5 | 860 | 15 | 1,928,000 | 622,000 | 4,727,000 | 498,000 |
| **Intermittent Milkings*** | | | | | | | | |
| 7–15 AM | 135 | 0 | | | 1,866,000 | 746,000 | | |
| 16 AM | 60 | 30 | | | 1,990,000 | 1,244,000 | | |
| 17 AM | 195 | 20 | | | 1,057,000 | 684,000 | | |
| 19 AM | 35 | 75 | | | 7,588,000 | 995,000 | | |
| 22 AM | 85 | 95 | | | 8,895,000 | 3,110,000 | | |
| 26 AM | 20 | 5 | | | 14,866,000 | 6,282,000 | | |
| **Post-parturition**** | | | | | | | | |
| 10–17 AM | 395 | 10 | 1700 | 1350 | 12,253,000 | 9,019,000 | 154,256,000 | 13,808,000 |
| PM | 150 | 15 | 750 | 2120 | 6,096,000 | 5,660,000 | 47,272,000 | 4,105,000 |
| 18 AM | 55 | 275 | 105 | 150 | 2,488,000 | 2,364,000 | 33,531,000 | 2,861,000 |
| PM | 110 | 0 | 113 | 5 | 3,794,000 | 1,928,000 | 58,219,000 | 4,727,000 |
| 23 PM | 375 | 10 | 20 | 15 | 560,000 | 249,000 | 2,612,000 | 249,000 |
| 24 AM | 95 | 5 | 130 | 10 | 684,000 | 435,000 | 11,679,000 | 249,000 |
| 11–30 PM | 75 | 35 | 5 | 60 | 871,000 | 5,536,000 | 684,000 | 249,000 |
| 12– 1 AM | 2370 | 90 | 15 | 15 | 311,000 | 933,000 | 435,000 | 373,000 |

* Milking of left half discontinued 7–14 to 7–26–30.

** Calved 10–17–30.

Legend: RF—Right fore quarter.
RH—Right hind quarter.
LF—Left fore quarter.
LH—Left hind quarter.

As shown in table 2, there was a slightly, but scarcely significant, lower average bacterial count during the period of incomplete milking but a noticeably higher cell count. The average bacterial count during the period of incomplete intermittent milking was nearly twice as high as the counts in

TABLE 2

*Effect of incomplete and intermittent incomplete milking on the bacterial and cell counts of milk*

Cow 173

| PERIOD | NUMBER OF MILKINGS | AVERAGE PRODUCTION PER MILKING | AVERAGE BACTERIAL COUNT* | AVERAGE CELL COUNT* |
|---|---|---|---|---|
| | | *gms.* | *per cc.* | *per cc.* |
| Preliminary | 3 | 1,792 | 684 | 1,584,000 |
| Incomplete milking | 8 | 1,249 | 613 | 2,394,000 |
| Intermittent, incomplete milking | 6 | 735 | 1,335 | 7,785,000 |

* All average bacterial and cell counts in this and subsequent tables are calculated by multiplying individual counts by the amount of milk obtained at the corresponding milking, adding these products and dividing by the total milk production for the period concerned.

the preliminary or incomplete milking periods preceding, while the cell counts showed marked increases. The differences in bacterial count during the three periods are no more extreme than normal fluctuations observed during preliminary milkings. The general upward trend in the cell counts is more significant. The incomplete and intermittent milking apparently had a more pronounced effect on the cell count than on the bacterial content.

### EFFECT OF INCOMPLETE, INTERMITTENT MILKING AND COMPLETE CESSATION OF MILKING

Cow 146.—Counts were made on four complete preliminary milkings at 12 hour intervals. Following this period, the milking of the left half of the udder was discontinued for nineteen days, while the right half was milked incompletely and intermittently. With the right half, approximately one-half of the milk was removed every 24 hours for ten days, then at intervals of 2 days, 3 days, and 4 days.

The results of these trials are given in table 3. It will be observed that the average bacterial and cell counts of the milk during the preliminary period were unusually low. There was a gradual increase in the bacterial count of the milk from the right half of the udder which was being milked incompletely and intermittently. The bacterial count of the milk drawn from the left half of the udder which had not been milked for nineteen days was practically identical with that from the right half at the same time. As

TABLE 3

*Effect of intermittent, incomplete milking and complete cessation of milking on the bacterial and cell counts of milk*

Cow 146

| PERIOD | NUMBER OF MILKINGS | AVERAGE PRODUCTION PER MILKING | | AVERAGE BACTERIAL COUNT | | AVERAGE CELL COUNT | |
|---|---|---|---|---|---|---|---|
| | | Right half** | Left half | Right half | Left half | Right half | Left half |
| | | *gms.* | *gms.* | *per cc.* | *per cc.* | *per cc.* | *per cc.* |
| Preliminary | 4 | 2,144 | 1,658 | 2 | 0 | 235,000 | 220,000 |
| Intermittent incomplete | 12 | 1,535 | * | 48 | * | 2,286,000 | * |
| Resumption of regular milking | 1 | 533 | 280 | 518 | 515 | 20,075,000 | 8,913,000 |

* No milkings.

** Right half } indicate right and left half of udder in this and subsequent
Left half } tables.

was observed with cow 173, there was an increase in cell count as a result of incomplete and intermittent milking, and this was more marked than that occurring when milking was discontinued entirely during the same period. The results indicate that one might expect increases in cell and bacterial counts because of such practices but that there was no advantage in partial milking. Better results appeared to be obtained when milking was stopped abruptly.

EFFECT OF INTERMITTENT AND COMPLETE CESSATION OF MILKING

Cows 70, 73, 138, 155, 536, and 538.—All cows were milked every twelve hours for two days during the preliminary period with the exception of cow 155 which was milked for three days. Then the milking of the left half of the udder was discontinued until the next parturition. The right half of the udder was milked completely every twenty-four hours for three days and then as follows:

Cow 70—at intervals of 2, 3, 4, and 11 days. She freshened 28 days after the last milking.

Cow 73—at intervals of 2, 3, 4, and 7 days. She freshened 33 days after the last milking.

Cow 138—at intervals of 2, 3, and 8 days. She freshened 43 days after the last milking.

Cow 155—at intervals of 2, 3, 4, and 7 days. She freshened 76 days after the last milking.

Cow 536—at intervals of 2, 3, 4, and 7 days. She freshened 47 days after the last milking.

TABLE 4

*Effect of intermittent and complete cessation of milking on the bacterial and cell counts of milk*

| COW NO. | PERIOD | NUMBER OF MILKINGS | AVERAGE PRODUCTION PER MILKING | | AVERAGE BACTERIAL COUNT | | AVERAGE CELL COUNT | |
|---|---|---|---|---|---|---|---|---|
| | | | Right half | Left half | Right half | Left half | Right half | Left half |
| | | | *gms.* | *gms.* | *per cc.* | *per cc.* | *per cc.* | *per cc.* |
| 70 | Preliminary | 4 | 1,533 | 1,443 | 442 | 696 | 1,210,000 | 1,419,000 |
| | Intermittent | 7 | 1,847 | * | 224 | * | 4,681,000 | * |
| | Colostral | 4 | 3,790 | 3,815 | 349 | 392 | 1,684,000 | 8,593,000 |
| | 10th day post-parturition | 2 | 4,765 | 4,705 | 746 | 532 | 538,000** | 62,000*** |
| 73 | Preliminary | 4 | 1,768 | 1,566 | 1,907 | 16 | 432,000 | 165,000 |
| | Intermittent | 7 | 1,887 | * | 2,509 | * | 16,272,000 | * |
| | Colostral | 4 | 1,645 | 1,765 | 33,848 | 40,226 | 21,585,000 | 17,000,000 |
| | 10th day post-parturition | 2 | 3,290 | 3,410 | 886 | 427 | 1,643,000 | 1,922,000 |
| 138 | Preliminary | 4 | 1,661 | 1,093 | 4 | 5,614 | 354,000 | 4,029,000 |
| | Intermittent | 6 | 2,579 | * | 6 | * | 1,393,000 | * |
| | Colostral | 4 | 3,089 | 3,443 | 20 | 2,718 | 2,841,000 | 4,403,000 |
| | 10th day post-parturition | 4 | 4,557 | 4,795 | 26 | 5,743 | 143,000 | 489,000 |
| 155 | Preliminary | 6 | 1,259 | 1,239 | 66 | 643 | 858,000 | 1,766,000 |
| | Intermittent | 6 | 2,788 | * | 55 | * | 3,269,000 | * |
| | Colostral | 4 | 2,263 | 1,538 | 124 | 875 | 5,246,000 | 26,197,000 |
| | 10th day post-parturition | 4 | 3,988 | 2,658 | 546 | 30 | 2,027,000 | 374,000 |
| 536 | Preliminary | 4 | 1,360 | 936 | 251 | 393 | 838,000 | 817,000 |
| | Intermittent | 7 | 2,697 | * | 338 | * | 1,969,000 | * |
| | Colostral | 4 | 2,049 | 2,330 | 292 | 33 | 6,375,000 | 5,054,000 |
| | 10th day post parturition | 4 | 2,118 | 2,395 | 501 | 354 | 3,065,000 | 409,000 |
| 538 | Preliminary | 4 | 1,825 | 1,636 | 35 | 28 | 471,000 | 557,000 |
| | Intermittent | 7 | 2,316 | * | 62 | * | 3,039,000 | * |
| | Colostral | 4 | 2,314 | 2,340 | 25 | 40 | 1,315,000*** | 3,029,000*** |
| | 10th day post-parturition | 4 | 3,445 | 3,345 | 58 | 62 | 334,000** | 431,000** |

*—No milkings.

**—One sample missing.

***—Two samples missing.

Cow 538—at intervals of 2, 3, 4, and 7 days. She freshened 47 days after the last milking.

The data are given in table 4. The "colostral" period was designated arbitrarily as the first four milkings following parturition. The "post-parturition" period was selected as the tenth day after parturition.

The results indicate that there was no advantage in continuing to milk the cow intermittently in an attempt to dry her up, at least as far as the bacterial and cell counts are concerned.

In most cases the bacterial counts were lower during the "colostral" period than they were during the "post-parturition" period. The converse was true for the cell counts, suggesting a protective effect of the cells in the colostrum.

There appeared to be no tendency for bacterial or cell counts to be higher in the next lactation period following complete cessation of milking as practiced on the left halves of the udders of these six cows.

### EFFECT OF COMPLETE CESSATION OF MILKING

Cow 167—Four preliminary milkings were made 12 hours apart. Milking was discontinued until parturition 32 days later.

Cow 382—Three preliminary milkings were made twelve hours apart. Milking was then discontinued until parturition 141 days later.

Cow 542—Four preliminary milkings were made 12 hours apart. Milking was then discontinued until parturition 122 days later.

Following parturition milkings were made at regular 12 hour intervals. The data for these trials are presented in table 5. It is evident that complete cessation of milking did not lead to higher bacterial counts in the subsequent lactation period. There is an apparent tendency for higher cell counts following parturition, but the differences are not marked, and in fact,

TABLE 5

*Effect of complete cessation of milking on the bacterial and cell counts of milk*

| COW NO. | AVERAGE PRODUCTION PER MILKING | | | | AVERAGE BACTERIAL COUNTS | | AVERAGE CELL COUNTS | |
|---|---|---|---|---|---|---|---|---|
| | PRELIMINARY PERIOD | | POST-PARTURITION PERIOD | | PRELIMINARY PERIOD | POST-PARTURITION | PRELIMINARY PERIOD | POST-PARTURITION |
| | Number of milkings | Production | Number of milkings | Production | | | | |
| | | *gms.* | | *gms.* | *per cc.* | *per cc.* | *per cc.* | *per cc.* |
| 167 | 4 | 1538 | 6 | 5008 | 727 | 610 | 1,093,000 | 2,456,000 |
| 382 | 3 | 1567 | 4 | 7392 | 63 | 19 | 2,660,000 | 1,622,000 |
| 542 | 4 | 2459 | 6 | 4618 | 1166 | 382 | 1,822,000 | 5,545,000 |

TABLE 6

*Effect of suspended milking on the bacterial and cell counts of milk*

| COW NO. | DAYS SUSPENDED | PERIOD | NUMBER OF MILKINGS | AVERAGE PRODUCTION PER MILKING | | AVERAGE BACTERIAL COUNT | | AVERAGE CELL COUNT | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Right half | Left half | Right half | Left half | Right half | Left half |
| | | | | *gms.* | *gms.* | *per cc.* | *per cc.* | *per cc.* | *per cc.* |
| 392 | | Preliminary | 3 | 2,347 | 2,427 | 913 | 1,215 | * | * |
| | 16 | Resumption | 1 | 300 | 420 | 16,828 | 1,717 | * | * |
| 410 | | Preliminary | 4 | 890 | 1,905 | 91 | 202 | 639,000 | 374,000 |
| | 6 | Resumption 1 | 1 | 790 | 2,010 | 44 | 372 | 27,977,000 | 13,181,000 |
| | 11 | Resumption 2 | 1 | 670 | 1,130 | 903 | 18 | 20,133,000 | 24,942,000 |
| 548 | | Preliminary | 2 | 890 | 848 | 486 | 41 | 462,000 | 448,000 |
| | 10 | Resumption | 1 | 200 | 300 | 3,863 | 20 | 9,791,000 | 10,764,000 |

*—No counts made.

are no greater than the ordinary fluctuations observed from milking to milking in the preliminary period.

EFFECT OF SUSPENDED MILKING

Cow 392—Three preliminary milkings were made at 12 hour intervals. Milking was then suspended for 16 days after which it was resumed.

Cow 410—Four preliminary milkings were made at 12 hour intervals. Milking was then suspended for 6 days after which one milking was made, followed by another period of suspension for 11 days after which one milking was made.

Cow 548—Two preliminary milkings were made at 12 hour intervals. Milking was then suspended for 10 days after which it was resumed.

Table 6 gives the results of these trials. It is shown that the bacterial counts of samples taken after a period of suspended milking were in most cases somewhat higher than in the normal milking periods. In most cases these differences were not marked. On the other hand, the cell counts were decidedly higher as a result of suspended milking. It would seem in the light of this and previous experiments that if milking is suspended sometime during the lactation period it should not be resumed until the next parturition. In some of the quarters of the cows in this experiment there were definite signs of inflammation evidenced upon post-mortem examinations. Whether these involvements were the result of the suspended milking is not certain. However, certain quarters were not affected so that it is not unreasonable to believe that factors other than suspended milkings were to blame for these changes.

Cow 410.—This animal had aborted 51 days before she was due to calf. *Brucella abortus* had been isolated from the milk of all four quarters of the udder. At the time she was placed on the experiment she had been in milk 58 days. Following a preliminary period of five milkings at 12 hour intervals, no milk was drawn from the udder for 11½ days, after which time all quarters were milked dry. The left half was then milked regularly every 12 hours while milking was suspended in the right half for 9½ days longer, after which regular 12 hour milkings were resumed.

As shown in table 7, the suspension of milking brought about marked increases in cell counts following such practice. As the regular 12 hour milkings continued the cell counts gradually returned to normal in the left half but did not reach the level of the preliminary period in the right half, although there was a general downward trend.

BACTERIAL AND CELL COUNTS OF NORMAL MILK

The data obtained in the preliminary periods of the various trials reported previously have been assembled in tables 8 and 9 as a matter of rec-

TABLE 7

*The effect of suspended milking on cell counts of milk*

Cow 410

| DATE | RIGHT HALF OF UDDER | | LEFT HALF OF UDDER | |
|---|---|---|---|---|
| | Milk Yield | Cell Count | Milk Yield | Cell Count |
| | *gms.* | *per cc.* | *gms.* | *per cc.* |
| Preliminary Period | | | | |
| (1930) 3–25 PM | 1675 | 1,347,000 | 1125 | 3,467,000 |
| 26 AM | 2590 | 382,000 | 2305 | 867,000 |
| PM | 2085 | 1,067,000 | 2435 | 1,990,000 |
| 27 AM | 2365 | 671,000 | 1855 | 1,967,000 |
| PM | 2290 | 807,000 | 1970 | 1,129,000 |
| Milking suspended until | | | | |
| 4– 8 AM | 1200 | 10,198,000 | 1251 | 18,384,000 |
| PM* | | | 593 | 39,406,000 |
| 9 AM | | | 490 | 59,733,000 |
| PM | | | 620 | 55,219,000 |
| 10 AM | | | 805 | 34,599,000 |
| PM | | | 820 | 25,663,000 |
| 11 AM | | | 965 | 18,696,000 |
| PM | | | 1030 | 15,687,000 |
| 12 AM | | | 1175 | 13,625,000 |
| PM | | | 1035 | 13,821,000 |
| 13 AM | | | 1190 | 15,271,000 |
| PM | | | 1005 | 10,581,000 |
| 14 AM | | | 935 | 12,743,000 |
| PM | | | 1010 | 9,003,000 |
| 15 AM | | | 1170 | 6,804,000 |
| PM | | | 1135 | 6,884,000 |
| 16 AM | | | 1414 | 5,150,000 |
| PM | | | 1355 | 6,418,000 |
| 17 AM | | | 1615 | 5,721,000 |
| PM | 1145 | 15,129,000 | 1345 | 5,603,000 |
| 18 AM | 375 | 35,304,000 | 1385 | 3,777,000 |
| PM | 455 | 21,988,000 | 1615 | 4,448,000 |
| 19 AM | 350 | 17,194,000 | 1545 | 7,272,000 |
| PM | 440 | 14,648,000 | 1620 | 5,986,000 |
| 20 AM | 380 | 18,137,000 | 1725 | 4,161,000 |
| PM | 495 | 15,895,000 | 1750 | 3,049,000 |
| 21 AM | 480 | 13,844,000 | 1645 | 2,825,000 |
| PM | 465 | 15,515,000 | 1640 | 1,999,000 |
| 22 AM | 540 | 8,657,000 | 1855 | 2,622,000 |
| PM | 620 | 8,328,000 | 1835 | ** |
| 23 AM | 565 | 9,124,000 | 1855 | ** |
| PM | 595 | 7,487,000 | 1935 | ** |
| 24 AM | 630 | 8,024,000 | 1870 | ** |
| PM | 595 | 8,208,000 | 1865 | ** |
| 25 AM | 570 | 8,859,000 | 1665 | ** |
| 5– 5 PM | 865 | 4,253,000 | 1810 | 1,670,000 |
| 6 AM | 935 | 5,407,000 | 2245 | 1,729,000 |

*—Right half—milking suspended for succeeding nine days.

**—No counts made.

ord. The samples are representative of milk, drawn aseptically from normal cows.

Table 8 gives a distribution analysis of the bacterial counts as well as the average for each and all quarters. The majority of the samples gave counts below 500 per cubic centimeter.

TABLE 8

*Bacterial content of fresh milk drawn aseptically from normal cows*
(284 samples)

| BACTERIAL COUNT | DISTRIBUTION OF SAMPLES ACCORDING TO BACTERIAL COUNTS | | | | |
|---|---|---|---|---|---|
| | Right fore quarter | Right hind quarter | Left fore quarter | Left hind quarter | All quarters |
| *per cc.* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 0 | 14.1 | 25.3 | 19.7 | 12.7 | 17.9 |
| 1– 99 | 35.2 | 36.6 | 38.0 | 38.0 | 36.9 |
| 100– 499 | 21.1 | 19.7 | 19.7 | 15.5 | 19.0 |
| 500– 999 | 5.6 | 7.0 | 2.8 | 14.1 | 7.4 |
| 1,000– 2,499 | 12.7 | 9.9 | 7.0 | 11.3 | 10.2 |
| 2,500– 4,999 | 9.9 | 1.5 | 11.3 | 4.2 | 6.7 |
| 5,000– 9,999 | 1.4 | 0.0 | 1.5 | 1.4 | 1.1 |
| 10,000–16,500 | 10.0 | 0.0 | 0.0 | 2.8 | 0.8 |
| | Average Bacterial Counts | | | | |
| | *per cc.* 668 | *per cc.* 311 | *per cc.* 693 | *per cc.* 958 | *per cc.* 658 |

TABLE 9

*Cell content of fresh milk drawn aseptically from normal cows*

| CELL COUNT | DISTRIBUTION OF SAMPLES ACCORDING TO CELL COUNTS | | | | |
|---|---|---|---|---|---|
| | Right fore quarter | Right hind quarter | Left fore quarter | Left hind quarter | All quarters |
| *per cc.* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 100,000– 499,000 | 40.8 | 29.6 | 42.3 | 38.0 | 37.7 |
| 500,000– 999,000 | 19.7 | 42.3 | 29.6 | 15.5 | 26.8 |
| 1,000,000– 2,499,000 | 24.0 | 16.9 | 11.2 | 28.2 | 20.1 |
| 2,500,000– 4,999,000 | 9.9 | 9.9 | 16.9 | 12.7 | 12.3 |
| 5,000,000– 9,999,000 | 5.6 | 1.3 | 0.0 | 4.2 | 2.8 |
| 10,000,000–13,373,000 | 0.0 | 0.0 | 0.0 | 1.4 | 0.3 |
| | Average Cell Counts | | | | |
| | *per cc.* 1,264,000 | *per cc.* 1,091,000 | *per cc.* 1,115,000 | *per cc.* 1,540,000 | *per cc.* 1,252,000 |

The cell counts are similarly reported in table 9. The majority of counts are below 1,000,000 per cubic centimeter. The average bacterial and cell counts are in reasonably close agreement with those reported by other investigators.

## DISCUSSION OF RESULTS

The data collected in these studies indicate that the stopping of milk secretion by complete cessation of milking had no marked effect on the bacterial content of milk obtained in the subsequent lactation period. This method of drying up cows was as satisfactory as the commonly practiced intermittent and incomplete method. In fact, these results appear to be in favor of complete cessation of milking.

The cells observed in the milk during the drying up process were large mono-nuclear cells, similar to those described by Varrier-Jones. Normal milk contained a larger proportion of polynuclear cells.

It is recognized that it would be desirable to continue similar experiments on cows during several succeeding lactation periods to be certain as to the permanent effects on the bacterial and cell content of the milk as a result of various methods for drying up cows.

## SUMMARY

(1) Studies of the effect of various methods of drying up cows on the bacterial and cell content of the milk are reported.

(2) Incomplete, intermittent, incomplete intermittent milking and complete cessation of milking did not appear to have any marked effect on the bacterial or cell counts of the milk in subsequent lactation periods.

(3) Intermittent and incomplete milking usually resulted in somewhat higher bacterial and cell counts during such periods but apparently did not produce permanent effects.

(4) Complete cessation of milking appeared to give the most satisfactory results.

FIG. 1. PHOTOGRAPH SHOWING METHOD OF OBTAINING ASEPTIC SAMPLE OF MILK. THE LINEN CLOTH WAS SOAKED IN CHLORAMINE-T SOLUTION BEFORE USE. A SMALL HOLE IN THE CLOTH ALLOWED FOR THE INTRODUCTION OF THE TEAT, SO THAT THE HAND WAS KEPT U[illegible]R THE CLOTH DURING THE MILKING IN SUCH A WAY THAT CONTAMINATION WAS AVOIDED FROM THE EXTERIOR OF THE COW AND THE ATMOSPHERE.

(5) Bacterial counts were usually somewhat lower during the first few milkings following parturition than they were during periods ten days following calving.

(6) Bacterial counts were slightly higher immediately following periods of suspended milking, while the cell counts were decidedly higher.

(7) The cell counts were generally higher during the "colostral" period and then became lower as the period of lactation advanced.

(8) The average bacterial count of 284 samples of normal, aseptically drawn milk was 658 while the average cell count was 1,252,000 per cubic centimeter.

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# American Dairy Science Association Announcements

## NEW OFFICERS

The Association elected H. C. Jackson, in charge of the Dairy Department at the University of Wisconsin, President for the current year, and reelected R. B. Stoltz, in charge of the Department of Dairy Technology at Ohio State University, as Vice-President.

All officers of the sections were changed this year, except for Dairy Economics, and the new officers are given on the directory page.

### Meeting of Eastern Section

The Eastern Division held its eleventh annual meeting on September 18–20, 1932, in the Hotel Clinton, Springfield, Massachusetts.

Chairman E. S. Guthrie, of New York, presided at the various meetings. The first session was devoted to production problems. Papers were presented by V. A. Rice, of Massachusetts, on bull indexes; by R. C. Foley, of Massachusetts, on pastures; by J. M. Fuller, of New Hampshire, on economical management of herds; by E. S. Savage, of New York, on effect of protein intake on milk production. The report of J. W. Bartlett, of New Jersey, of committee on Teaching Methods was given by C. B. Bender.

The second session was devoted entirely to dairy manufactures. A paper was presented on relationship of gelatin and homogenization pressures to texture of ice cream by T. A. Lyons, of Connecticut; on a milk survey by H. C. Moore, of New Hampshire; on effect of aging gelatin on certain of its properties by W. S. Mueller, of Massachusetts; on cultured cream by E. S. Guthrie, of New York; on abnormal milk flavors by Frandsen and De Pew, of Massachusetts.

The principal address at the banquet was given by William H. Pew, of Pine Plains, New York, on marketing of agricultural products. The attendance was 82.

There were 7 teams in the Dairy Products Judging contest. Vermont won first, Connecticut second, and Massachusetts third. In the Livestock Judging there were 6 teams. In all classes Pennsylvania won first, Connecticut second, and New Hampshire third. In Dairy Cattle Judging there were 11 teams. Maryland was first, Cornell second, and New Hampshire third.